WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

VOLUME 61
SCHISTOSOMES, LIVER FLUKES AND *HELICOBACTER PYLORI*

1994
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F R A N C E

WORLD HEALTH ORGANIZATION

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IARC MONOGRAPHS

ON THE

EVALUATION OF CARCINOGENIC RISKS TO HUMANS

Schistosomes, Liver Flukes and Helicobacter pylori

VOLUME 61

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Carcinogenic Risks to Humans,
which met in Lyon,

7–14 June 1994

1994

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. In 1980 and 1986, the programme was expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures and other agents.

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields; and to indicate where additional research efforts are needed.

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NOTE TO THE READER

The term 'carcinogenic risk' in the *IARC Monographs* series is taken to mean the probability that exposure to an agent will lead to cancer in humans.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a monograph does not mean that it is not carcinogenic.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Carcinogen Identification and Evaluation, so that corrections can be reported in future volumes.

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Lyon, 7–14 June 1994

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PREAMBLE

IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS[1]

PREAMBLE

1. BACKGROUND

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme to evaluate the carcinogenic risk of chemicals to humans and to produce monographs on individual chemicals. The *Monographs* programme has since been expanded to include consideration of exposures to complex mixtures of chemicals (which occur, for example, in some occupations and as a result of human habits) and of exposures to other agents, such as radiation and viruses. With Supplement 6 (IARC, 1987a), the title of the series was modified from *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* to *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, in order to reflect the widened scope of the programme.

The criteria established in 1971 to evaluate carcinogenic risk to humans were adopted by the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monographs* series. Those criteria were subsequently updated by further ad-hoc working groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987b, 1988, 1991a; Vainio *et al.*, 1992).

2. OBJECTIVE AND SCOPE

The objective of the programme is to prepare, with the help of international working groups of experts, and to publish in the form of monographs, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* may also indicate where additional research efforts are needed.

The *Monographs* represent the first step in carcinogenic risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that certain exposures could alter the incidence of cancer in humans. The second step is quantitative risk estimation. Detailed, quantitative evaluations of epidemiological data may be made in the *Monographs*, but without extrapolation beyond the range of the data

[1]This project is supported by PHS Grant No. 5-UO1 CA33193-13 awarded by the US National Cancer Institute, Department of Health and Human Services. Since 1986, the programme has also been supported by the European Commission.

available. Quantitative extrapolation from experimental data to the human situation is not undertaken.

The term 'carcinogen' is used in these monographs to denote an exposure that is capable of increasing the incidence of malignant neoplasms; the induction of benign neoplasms may in some circumstances (see p. 26) contribute to the judgement that the exposure is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991a; Vainio *et al.*, 1992; see also pp. 32–34).

The *Monographs* may assist national and international authorities in making risk assessments and in formulating decisions concerning any necessary preventive measures. The evaluations of IARC working groups are scientific, qualitative judgements about the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which regulatory measures may be based. Other components of regulatory decisions may vary from one situation to another and from country to country, responding to different socioeconomic and national priorities. **Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments and/or other international organizations.**

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of a wide range of human exposures. A users' survey, made in 1988, indicated that the *Monographs* are consulted by various agencies in 57 countries. Each volume is generally printed in 4000 copies for distribution to governments, regulatory bodies and interested scientists. The *Monographs* are also available *via* the Distribution and Sales Service of the World Health Organization.

3. SELECTION OF TOPICS FOR MONOGRAPHS

Topics are selected on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some evidence or suspicion of carcinogenicity. The term 'agent' is used to include individual chemical compounds, groups of related chemical compounds, physical agents (such as radiation) and biological factors (such as viruses). Exposures to mixtures of agents may occur in occupational exposures and as a result of personal and cultural habits (like smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. The IARC surveys of chemicals being tested for carcinogenicity (IARC, 1973–1992) and directories of on-going research in cancer epidemiology (IARC, 1976–1994) often indicate those exposures that may be scheduled for future meetings. Ad-hoc working groups convened by IARC in 1984, 1989, 1991 and 1993 gave recommendations as

to which agents should be evaluated in the *IARC Monographs* series (IARC, 1984, 1989, 1991b, 1993).

As significant new data on subjects on which monographs have already been prepared become available, re-evaluations are made at subsequent meetings, and revised monographs are published.

4. DATA FOR MONOGRAPHS

The *Monographs* do not necessarily cite all the literature concerning the subject of an evaluation. Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to biological and epidemiological data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the working groups. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see pp. 26 *et seq.*). In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, unpublished sources of information may be used.

5. THE WORKING GROUP

Reviews and evaluations are formulated by a working group of experts. The tasks of the group are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanism of action; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans.

Working Group participants who contributed to the considerations and evaluations within a particular volume are listed, with their addresses, at the beginning of each publication. Each participant who is a member of a working group serves as an individual scientist and not as a representative of any organization, government or industry. In addition, nominees of national and international agencies and industrial associations may be invited as observers.

6. WORKING PROCEDURES

Approximately one year in advance of a meeting of a working group, the topics of the monographs are announced and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as BIOSIS, Chemical Abstracts, CANCERLIT, MEDLINE and TOXLINE—including EMIC and ETIC for data on genetic and related effects and reproductive and developmental effects, respectively.

For chemicals and some complex mixtures, the major collection of data and the preparation of first drafts of the sections on chemical and physical properties, on analysis, on

production and use and on occurrence are carried out under a separate contract funded by the US National Cancer Institute. Representatives from industrial associations may assist in the preparation of sections on production and use. Information on production and trade is obtained from governmental and trade publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available because their publication could disclose confidential information. Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants, or is used by IARC staff, to prepare sections for the first drafts of monographs. The first drafts are compiled by IARC staff and sent, prior to the meeting, to all participants of the Working Group for review.

The Working Group meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, edited and prepared for publication. The aim is to publish monographs within six months of the Working Group meeting.

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study, directly impinging on its interpretation, should be brought to the attention of the reader, a comment is given in square brackets.

7. EXPOSURE DATA

Sections that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are included at the beginning of each monograph.

Most monographs on individual chemicals, groups of chemicals or complex mixtures include sections on chemical and physical data, on analysis, on production and use and on occurrence. In monographs on, for example, physical agents, occupational exposures and cultural habits, other sections may be included, such as: historical perspectives, description of an industry or habit, chemistry of the complex mixture or taxonomy. Monographs on biological agents have sections on structure and biology, methods of detection, epidemiology of infection and clinical disease other than cancer.

For chemical exposures, the Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name and the IUPAC Systematic Name are recorded; other synonyms are given, but the list is not necessarily comprehensive. For biological agents, taxonomy and structure are described, and the degree of variability is given, when applicable.

Information on chemical and physical properties and, in particular, data relevant to identification, occurrence and biological activity are included. For biological agents, mode of replication, life cycle, target cells, persistence and latency and host response are given. A

description of technical products of chemicals includes trades names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

The purpose of the section on analysis or detection is to give the reader an overview of current methods, with emphasis on those widely used for regulatory purposes. Methods for monitoring human exposure are also given, when available. No critical evaluation or recommendation of any of the methods is meant or implied. The IARC publishes a series of volumes, *Environmental Carcinogens: Methods of Analysis and Exposure Measurement* (IARC, 1978–93), that describe validated methods for analysing a wide variety of chemicals and mixtures. For biological agents, methods of detection and exposure assessment are described, including their sensitivity, specificity and reproducibility.

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents which do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided. In addition, methods of synthesis used in past and present commercial production and different methods of production which may give rise to different impurities are described.

Data on production, international trade and uses are obtained for representative regions, which usually include Europe, Japan and the USA. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. In the case of mixtures, industries, occupations or processes, information is given about all agents present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with time and place. For biological agents, the epidemiology of infection is described.

Statements concerning regulations and guidelines (e.g., pesticide registrations, maximal levels permitted in foods, occupational exposure limits) are included for some countries as indications of potential exposures, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccines and therapy, are described.

8. STUDIES OF CANCER IN HUMANS

(*a*) *Types of studies considered*

Three types of epidemiological studies of cancer contribute to the assessment of carcinogenicity in humans—cohort studies, case–control studies and correlation (or

ecological) studies. Rarely, results from randomized trials may be available. Case series and case reports of cancer in humans may also be reviewed.

Cohort and case–control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence/mortality in those exposed to incidence/mortality in those not exposed) as the main measure of association.

In correlation studies, the units of investigation are usually whole populations (e.g., in particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent, mixture or exposure circumstance under study. Because individual exposure is not documented, however, a causal relationship is less easy to infer from correlation studies than from cohort and case–control studies. Case reports generally arise from a suspicion, based on clinical experience, that the concurrence of two events—that is, a particular exposure and occurrence of a cancer—has happened rather more frequently than would be expected by chance. Case reports usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure. The uncertainties surrounding interpretation of case reports and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, relevant case reports or correlation studies may add materially to the judgement that a causal relationship is present.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed by working groups. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(*b*) *Quality of studies considered*

The *Monographs* are not intended to summarize all published studies. Those that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when they provide the only data available. Their inclusion does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of the study description.

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. By 'bias' is meant the operation of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between disease and an agent, mixture or exposure circumstance. By 'confounding' is meant a situation in which the relationship with disease is made to appear stronger or to appear weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. In evaluating the extent to which these factors have been minimized in an individual study, working groups consider a number of aspects of design and analysis as described in the report of the study. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the

reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

Firstly, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Secondly, the authors should have taken account in the study design and analysis of other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may be more appropriate than those with national rates. Internal comparisons of disease frequency among individuals at different levels of exposure should also have been made in the study.

Thirdly, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. The methods used should preferably have been the generally accepted techniques that have been refined since the mid-1970s. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(*c*) *Inferences about mechanism of action*

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure and time since exposure ceased, are reviewed and summarized when available. The analysis of temporal relationships can be useful in formulating models of carcinogenesis. In particular, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although at best they allow only indirect inferences about the mechanism of action. Special attention is given to measurements of biological markers of carcinogen exposure or action, such as DNA or protein adducts, as well as markers of early steps in the carcinogenic process, such as proto-oncogene mutation, when these are incorporated into epidemiological studies focused on cancer incidence or mortality. Such measurements may allow inferences to be made about putative mechanisms of action (IARC, 1991a; Vainio *et al.*, 1992).

(*d*) *Criteria for causality*

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent,

mixture or exposure circumstance in question is carcinogenic for humans. In making their judgement, the Working Group considers several criteria for causality. A strong association (i.e., a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that relative risks of small magnitude do not imply lack of causality and may be important if the disease is common. Associations that are replicated in several studies of the same design or using different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in amount of exposure), and results of studies judged to be of high quality are given more weight than those from studies judged to be methodologically less sound. When suspicion of carcinogenicity arises largely from a single study, these data are not combined with those from later studies in any subsequent reassessment of the strength of the evidence.

If the risk of the disease in question increases with the amount of exposure, this is considered to be a strong indication of causality, although absence of a graded response is not necessarily evidence against a causal relationship. Demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Although a carcinogen may act upon more than one target, the specificity of an association (i.e., an increased occurrence of cancer at one anatomical site or of one morphological type) adds plausibility to a causal relationship, particularly when excess cancer occurrence is limited to one morphological type within the same organ.

Although rarely available, results from randomized trials showing different rates among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, the judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first of all that the studies giving rise to it meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should be consistent with a relative risk of unity for any observed level of exposure and, when considered together, should provide a pooled estimate of relative risk which is at or near unity and has a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency for relative risk of cancer to increase with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained in this way from several epidemiological studies can apply only to the type(s) of cancer studied and to dose levels and intervals between first exposure and observation of disease that are the same as or less than those observed in all the studies. Experience with human cancer indicates that, in some cases, the period from first exposure to the development of clinical cancer is seldom less than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

9. STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

All known human carcinogens that have been studied adequately in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (aflatoxins, 4-aminobiphenyl, azathioprine, betel quid with tobacco, BCME and CMME (technical grade), chlorambucil, chlornaphazine, ciclosporin, coal-tar pitches, coal-tars, combined oral contraceptives, cyclophosphamide, diethylstilboestrol, melphalan, 8-methoxypsoralen plus UVA, mustard gas, myleran, 2-naphthylamine, nonsteroidal oestrogens, oestrogen replacement therapy/steroidal oestrogens, solar radiation, thiotepa and vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed the carcinogenicity in humans (Vainio *et al.*, 1994). Although this association cannot establish that all agents and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, **in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is sufficient evidence (see p. 31) of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans.** The possibility that a given agent may cause cancer through a species-specific mechanism which does not operate in humans (see p. 32) should also be taken into consideration.

The nature and extent of impurities or contaminants present in the chemical or mixture being evaluated are given when available. Animal strain, sex, numbers per group, age at start of treatment and survival are reported.

Other types of studies summarized include: experiments in which the agent or mixture was administered in conjunction with known carcinogens or factors that modify carcinogenic effects; studies in which the end-point was not cancer but a defined precancerous lesion; and experiments on the carcinogenicity of known metabolites and derivatives.

For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

An assessment is made as to the relevance to human exposure of samples tested in experimental animals, which may involve consideration of: (i) physical and chemical characteristics, (ii) constituent substances that indicate the presence of a class of substances, (iii) the results of tests for genetic and related effects, including genetic activity profiles, DNA adduct profiles, proto-oncogene mutation and expression and suppressor gene inactivation. The relevance of results obtained, for example, with animal viruses analogous to the virus being evaluated in the monograph must also be considered. They may provide biological and mechanistic information relevant to the understanding of the process of carcinogenesis in humans and may strengthen the plausibility of a conclusion that the biological agent that is being evaluated is carcinogenic in humans.

(*a*) *Qualitative aspects*

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route and schedule of exposure, species, strain, sex, age, duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the

spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

As mentioned earlier (p. 19), the *Monographs* are not intended to summarize all published studies. Those studies in experimental animals that are inadequate (e.g., too short a duration, too few animals, poor survival; see below) or are judged irrelevant to the evaluation are generally omitted. Guidelines for conducting adequate long-term carcinogenicity experiments have been outlined (e.g., Montesano *et al.*, 1986).

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was adequately monitored, particularly in inhalation experiments; (iii) whether the doses and duration of treatment were appropriate and whether the survival of treated animals was similar to that of controls; (iv) whether there were adequate numbers of animals per group; (v) whether animals of both sexes were used; (vi) whether animals were allocated randomly to groups; (vii) whether the duration of observation was adequate; and (viii) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, should be taken into account in the evaluation of tumour response.

When benign tumours occur together with and originate from the same cell type in an organ or tissue as malignant tumours in a particular study and appear to represent a stage in the progression to malignancy, it may be valid to combine them in assessing tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent or mixture induces only benign neoplasms that appear to be end-points that do not readily undergo transition to malignancy, it should nevertheless be suspected of being a carcinogen and it requires further investigation.

(*b*) *Quantitative aspects*

The probability that tumours will occur may depend on the species, sex, strain and age of the animal, the dose of the carcinogen and the route and length of exposure. Evidence of an increased incidence of neoplasms with increased level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Both DNA damage and increased cell division are important aspects of carcinogenesis, and cell proliferation is a strong determinant of dose–response relationships for some carcinogens (Cohen & Ellwein, 1990). Since many chemicals require metabolic activation before being converted into their reactive intermediates, both metabolic and pharmacokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship, as could saturation of processes such as DNA repair (Hoel *et al.*, 1983; Gart *et al.*, 1986).

(*c*) *Statistical analysis of long-term experiments in animals*

Factors considered by the Working Group include the adequacy of the information given for each treatment group: (i) the number of animals studied and the number examined

histologically, (ii) the number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto *et al.*, 1980; Gart *et al.*, 1986). When there is no difference in survival between control and treatment groups, the Working Group usually compares the proportions of animals developing each tumour type in each of the groups. Otherwise, consideration is given as to whether or not appropriate adjustments have been made for differences in survival. These adjustments can include: comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour is discovered), in the case where most differences in survival occur before tumours appear; life-table methods, when tumours are visible or when they may be considered 'fatal' because mortality rapidly follows tumour development; and the Mantel-Haenszel test or logistic regression, when occult tumours do not affect the animals' risk of dying but are 'incidental' findings at autopsy.

In practice, classifying tumours as fatal or incidental may be difficult. Several survival-adjusted methods have been developed that do not require this distinction (Gart *et al.*, 1986), although they have not been fully evaluated.

10. OTHER DATA RELEVANT TO AN EVALUATION OF CARCINOGENICITY AND ITS MECHANISMS

In coming to an overall evaluation of carcinogenicity in humans (see p. 28), the Working Group also considers related data. The nature of the information selected for the summary depends on the agent being considered.

For chemicals and complex mixtures of chemicals such as those in some occupational situations and involving cultural habits (e.g., tobacco smoking), the other data considered to be relevant are divided into those on absorption, distribution, metabolism and excretion; those on toxic effects; reproductive and developmental effects; and genetic and related effects.

Concise information is given on absorption, distribution (including placental transfer) and excretion in both humans and experimental animals. Kinetic factors that may affect the dose–response relationship, such as saturation of uptake, protein binding, metabolic activation, detoxification and DNA repair processes, are mentioned. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be of particular importance for extrapolation between species. Data are given on acute and chronic toxic effects (other than cancer), such as organ toxicity, increased cell proliferation, immunotoxicity and endocrine effects. The presence and toxicological significance of cellular receptors is described. Effects on reproduction, teratogenicity, fetotoxicity and embryotoxicity are also summarized briefly.

Tests of genetic and related effects are described in view of the relevance of gene mutation and chromosomal damage to carcinogenesis (Vainio *et al.*, 1992). The adequacy of the reporting of sample characterization is considered and, where necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests on p. 25. The available data are interpreted critically by

phylogenetic group according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and cell transformation. The concentrations employed are given, and mention is made of whether use of an exogenous metabolic system *in vitro* affected the test result. These data are given as listings of test systems, data and references; bar graphs (activity profiles) and corresponding summary tables with detailed information on the preparation of the profiles (Waters *et al.*, 1987) are given in appendices.

Positive results in tests using prokaryotes, lower eukaryotes, plants, insects and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information about the types of genetic effect produced and about the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g., gene mutations and chromosomal aberrations), while others are to a greater or lesser degree associated with genetic effects (e.g., unscheduled DNA synthesis). In-vitro tests for tumour-promoting activity and for cell transformation may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. A critical appraisal of these tests has been published (Montesano *et al.*, 1986).

Genetic or other activity manifest in experimental mammals and humans is regarded as being of greater relevance than that in other organisms. The demonstration that an agent or mixture can induce gene and chromosomal mutations in whole mammals indicates that it may have carcinogenic activity, although this activity may not be detectably expressed in any or all species. Relative potency in tests for mutagenicity and related effects is not a reliable indicator of carcinogenic potency. Negative results in tests for mutagenicity in selected tissues from animals treated *in vivo* provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence to rule out carcinogenicity of agents or mixtures that act through other mechanisms (e.g., receptor-mediated effects, cellular toxicity with regenerative proliferation, peroxisome proliferation) (Vainio *et al.*, 1992). Factors that may lead to misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al.*, 1986).

When available, data relevant to mechanisms of carcinogenesis that do not involve structural changes at the level of the gene are also described.

The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is evaluated by the same criteria as are applied to epidemiological studies of cancer.

Structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent are also described.

For biological agents—viruses, bacteria and parasites—other data relevant to carcinogenicity include descriptions of the pathology of infection, molecular biology (integration and expression of viruses, and any genetic alterations seen in human tumours) and other observations, which might include cellular and tissue responses to infection, immune response and the presence of tumour markers.

11. SUMMARY OF DATA REPORTED

In this section, the relevant epidemiological and experimental data are summarized. Only reports, other than in abstract form, that meet the criteria outlined on p. 19 are considered for evaluating carcinogenicity. Inadequate studies are generally not summarized: such studies are usually identified by a square-bracketed comment in the preceding text.

(*a*) *Exposures*

Human exposure to chemicals and complex mixtures is summarized on the basis of elements such as production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are given when available. Exposure to biological agents is described in terms of transmission, and prevalence of infection.

(*b*) *Carcinogenicity in humans*

Results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized.

(*c*) *Carcinogenicity in experimental animals*

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species and route of administration, it is stated whether an increased incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. If the agent or mixture produced tumours after prenatal exposure or in single-dose experiments, this is also indicated. Negative findings are also summarized. Dose–response and other quantitative data may be given when available.

(*d*) *Other data relevant to an evaluation of carcinogenicity and its mechanisms*

Data on biological effects in humans that are of particular relevance are summarized. These may include toxicological, kinetic and metabolic considerations and evidence of DNA binding, persistence of DNA lesions or genetic damage in exposed humans. Toxicological information, such as that on cytotoxicity and regeneration, receptor binding and hormonal and immunological effects, and data on kinetics and metabolism in experimental animals are given when considered relevant to the possible mechanism of the carcinogenic action of the agent. The results of tests for genetic and related effects are summarized for whole mammals, cultured mammalian cells and nonmammalian systems.

When available, comparisons of such data for humans and for animals, and particularly animals that have developed cancer, are described.

Structure–activity relationships are mentioned when relevant.

For the agent, mixture or exposure circumstance being evaluated, the available data on end-points or other phenomena relevant to mechanisms of carcinogenesis from studies in humans, experimental animals and tissue and cell test systems are summarized within one or more of the following descriptive dimensions:

(i) Evidence of genotoxicity (i.e., structural changes at the level of the gene): for example, structure–activity considerations, adduct formation, mutagenicity (effect on specific genes), chromosomal mutation/aneuploidy

(ii) Evidence of effects on the expression of relevant genes (i.e., functional changes at the intracellular level): for example, alterations to the structure or quantity of the product of a proto-oncogene or tumour suppressor gene, alterations to metabolic activation/-inactivation/DNA repair

(iii) Evidence of relevant effects on cell behaviour (i.e., morphological or behavioural changes at the cellular or tissue level): for example, induction of mitogenesis, compensatory cell proliferation, preneoplasia and hyperplasia, survival of premalignant or malignant cells (immortalization, immunosuppression), effects on metastatic potential

(iv) Evidence from dose and time relationships of carcinogenic effects and interactions between agents: for example, early/late stage, as inferred from epidemiological studies; initiation/promotion/progression/malignant conversion, as defined in animal carcinogenicity experiments; toxicokinetics

These dimensions are not mutually exclusive, and an agent may fall within more than one of them. Thus, for example, the action of an agent on the expression of relevant genes could be summarized under both the first and second dimension, even if it were known with reasonable certainty that those effects resulted from genotoxicity.

12. EVALUATION

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant data, the Working Group may assign the agent, mixture or exposure circumstance to a higher or lower category than a strict interpretation of these criteria would indicate.

(*a*) *Degrees of evidence for carcinogenicity in humans and in experimental animals and supporting evidence*

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency) nor to the mechanisms involved. A classification may change as new information becomes available.

An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of degree of evidence.

(i) *Carcinogenicity in humans*

The applicability of an evaluation of the carcinogenicity of a mixture, process, occupation or industry on the basis of evidence from epidemiological studies depends on the variability over time and place of the mixtures, processes, occupations and industries. The Working Group seeks to identify the specific exposure, process or activity which is considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

Inadequate evidence of carcinogenicity: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association, or no data on cancer in humans are available.

Evidence suggesting lack of carcinogenicity: There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent, mixture or exposure circumstance and any studied cancer at any observed level of exposure. A conclusion of 'evidence suggesting lack of carcinogenicity' is inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

(ii) *Carcinogenicity in experimental animals*

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Exceptionally, a single study in one species might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset.

Limited evidence of carcinogenicity: The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g., (a) the evidence of carcinogenicity is restricted to a single experiment; or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.

Inadequate evidence of carcinogenicity: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

Evidence suggesting lack of carcinogenicity: Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent or mixture is not carcinogenic. A conclusion of evidence suggesting lack of carcinogenicity is inevitably limited to the species, tumour sites and levels of exposure studied.

(*b*) *Other data relevant to the evaluation of carcinogenicity and its mechanisms*

Other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is then described. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and pharmacokinetics, physicochemical parameters and analogous biological agents.

Data relevant to mechanisms of the carcinogenic action are also evaluated. The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is assessed, using terms such as weak, moderate or strong. Then, the Working Group assesses if that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans come from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

For complex exposures, including occupational and industrial exposures, chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(*c*) *Overall evaluation*

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation of the carcinogenicity to humans of an agent, mixture or circumstance of exposure.

An evaluation may be made for a group of chemical compounds that have been evaluated by the Working Group. In addition, when supporting data indicate that other, related compounds for which there is no direct evidence of capacity to induce cancer in humans or in animals may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of compounds if the strength of the evidence warrants it.

The agent, mixture or exposure circumstance is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent, mixture or exposure circumstance is a matter of scientific judgement, reflecting the

strength of the evidence derived from studies in humans and in experimental animals and from other relevant data.

Group 1—The agent (mixture) is carcinogenic to humans.
The exposure circumstance entails exposures that are carcinogenic to humans.

This category is used when there is *sufficient evidence* of carcinogenicity in humans. Exceptionally, an agent (mixture) may be placed in this category when evidence in humans is less than sufficient but there is *sufficient evidence* of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity.

Group 2

This category includes agents, mixtures and exposure circumstances for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost sufficient, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents, mixtures and exposure circumstances are assigned to either group 2A (probably carcinogenic to humans) or group 2B (possibly carcinogenic to humans) on the basis of epidemiological and experimental evidence of carcinogenicity and other relevant data.

Group 2A—The agent (mixture) is probably carcinogenic to humans.
The exposure circumstance entails exposures that are probably carcinogenic to humans.

This category is used when there is *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals. In some cases, an agent (mixture) may be classified in this category when there is *inadequate evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent, mixture or exposure circumstance may be classified in this category solely on the basis of *limited evidence* of carcinogenicity in humans.

Group 2B—The agent (mixture) is possibly carcinogenic to humans.
The exposure circumstance entails exposures that are possibly carcinogenic to humans.

This category is used for agents, mixtures and exposure circumstances for which there is *limited evidence* of carcinogenicity in humans and less than *sufficient evidence* of carcinogenicity in experimental animals. It may also be used when there is *inadequate evidence* of carcinogenicity in humans but there is *sufficient evidence* of carcinogenicity in experimental animals. In some instances, an agent, mixture or exposure circumstance for which there is *inadequate evidence* of carcinogenicity in humans but *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group.

Group 3—The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents, mixtures and exposure circumstances for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals.

Exceptionally, agents (mixtures) for which the evidence of carcinogenicity is inadequate in humans but sufficient in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents, mixtures and exposure circumstances that do not fall into any other group are also placed in this category.

Group 4—The agent (mixture) is probably not carcinogenic to humans.

This category is used for agents or mixtures for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents or mixtures for which there is *inadequate evidence* of carcinogenicity in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group.

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GENERAL REMARKS

Several biological agents have been implicated in the development of human cancers. Following recomendations made by an advisory group which met in 1991 (IARC, 1991), the *IARC Monographs* programme was expanded to include consideration of exposure to or infection with biological agents such as viruses, bacteria and helminths. The fifty-ninth volume of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* evaluated human infections with hepatitis B virus, hepatitis C virus and hepatitis D virus. This sixty-first volume considers certain helminthic infections with schistosomes and liver flukes and bacterial infection with *Helicobacter pylori*.

Helminths are parasitic worms which differ from all other infectious agents in that they are larger and multicellular and are always located extracellularly in the mammalian host; they do not multiply in humans. The worms are highly aggregated in their distribution within infected communities, with a majority of worms being harboured by a minority of the infected population. It is this segment of the population that is at considerable risk of developing severe disease.

Schistosomiasis, which is considered in this volume, is widespread: it is estimated that 200 million people in at least 74 countries are infected (WHO, 1993). Five species of the *Schistosoma* trematodes cause disease globally: *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*. Only the first three, which account for the vast majority of schistosomal disease in humans, are considered here. A causal association between infection by *S. haematobium* and squamous-cell carcinoma of the urinary bladder was first postulated towards the beginning of this century (Goebel, 1905), and concern has been raised more recently about an association between infection with *S. mansoni* or *S. japonicum* and increased risk for cancers of the gastrointestinal tract (Inaba *et al.*, 1984; Chen & Mott, 1989).

About 17 million people in Europe and Asia are infected with certain liver flukes: with *Chlonorchis sinensis* in China, the Korean peninsula and Viet Nam; with *Opisthorchis viverrini* in Thailand and Laos; and with *O. felineus* in the Russian Federation and eastern Europe. Liver cancers were first described in association with infection with *O. felineus* and *C. sinensis* in clinical series nearly a century ago (Askanazy, 1900; Katsurada, 1900) and in association with *O. viverrini* 50 years later (Viranuvatti & Mettiyawongse, 1953).

The bacterium, *H. pylori*, first characterized and cultured in 1982 (Marshall, 1983; Warren, 1983), is the cause of most cases of chronic gastritis and duodenal ulcer. More than half of the world's population may be infected with *H. pylori*. The demonstration that atrophic gastritis is a precursor condition for gastric cancer led to the suggestion that this bacterium may be involved in the development of this cancer (Correa, 1992).

Chronic infection and inflammation contribute to the multistage carcinogenesis process by many different mechanisms. Several hypotheses have been proposed, which are briefly summarized below.

In response to infectious or inflammatory agents, inflammatory cells are activated to produce reactive oxygen and nitrogen species, which kill invading pathogens. These radicals can also damage macromolecules, including DNA, in adjacent normal tissues, resulting in the induction of mutations, DNA strand breaks and chromosomal aberrations. The increased rate of cell division and the decrease in efficiency of DNA repair in infected tissues may increase the rate of fixation of mutations. Inflammatory cells secrete various cytokines and enzymes, which may stimulate the growth of tumour cells (Ames & Gold, 1990; Cohen & Ellwein, 1990; Preston-Martin *et al.*, 1990).

A further mechanism by which bacteria and other parasites may contribute to carcinogenesis is the production of toxins and carcinogens. Bacteria and activated inflammatory cells can generate nitrosating agents from nitric oxide. Methylation damage to DNA has been reported in the urinary bladders of people infected with *S. haematobium* and in the livers of mice infected with *S. mansoni*. Alteration of the metabolism of carcinogens and endogenous substrates in the host may also play a role in the carcinogenic process.

In the evaluation of the biological agents considered in this volume, the same principles were used as in previous *Monographs* (see Preamble). Inferences on the role of these agents in the induction of neoplasia are based on the same criteria as those used for chemical compounds in the *Monographs* series. There are, however, minor differences in the nature of the evidence available for the assessment of the role played by infectious agents in the genesis of cancer. Unlike chemical exposures, for which the only available measure usually derives from memory or imperfect records, human exposure can be measured objectively, by the laboratory evaluation of biological specimens. Measures of exposure to infectious agents commonly have no time reference, however, and recent exposure cannot always be easily distinguished from exposure in the distant past. Moreover, the sensitivity and specificity of measures of exposure depend on the uniqueness of the agent and the host response.

In addition, with specific reference to helminthic infections, descriptive epidemiology assumes a rather more important role than it usually does. This is because exposure to helminths is sharply circumscribed geographically and demographically. Moreover, the malignancies suspected of originating with helminthic infection are in several cases rare. Thus, if the occurrence of an otherwise rare tumour is well circumscribed in terms of age, sex and geographical unit, it can be more easily compared to the analogous distribution of infection.

Studies of carcinogenesis in animals also assume a somewhat different role in the assessment of infectious agents than that of chemicals. While infected animals do develop tumours which are histologically very similar to those in infected humans, the 'dose' delivered is more difficult to control, since growth and, in the case of bacteria, replication occur in the host. Moreover, since the process of carcinogenesis may be dependent on the host reaction, the relationship between latency and the life span of the experimental animal becomes an important issue.

Various animal models have been used to study the carcinogenicity of these organisms and to explore their mechanisms of action. Although, as might have been expected for infectious biological agents, there were detectable differences in susceptibility to them among species and strains, what was truly remarkable was the extent to which the biological effects produced in the experimental animals occurred at the same sites and produced the same type of lesions as those seen in humans infected with these organisms. This target organ specificity was also observed in experiments in which tumour induction was produced by infection plus exposure to low doses of several chemical carcinogens. It may not be possible to expose animals to levels of infection that exceed human exposures to the degree that can often be done with chemicals. Therefore, it may not be possible with infectious agents like these to maximize their tumour response to a level that is detectable in bioassays of conventional size or to foreshorten the period to tumour occurrence, as is commonly done with chemical carcinogens. Even if the infection is an important factor in the development of tumours in people, these biological agents may not take all of the required steps in multistep carcinogenesis to yield neoplasms efficiently. Instead, they may be very effective in causing persistent injury and stimulating progressive cell proliferation at the site of infection. This process may efficiently achieve the first or several steps in transformation; later steps are traversed inefficiently with the organisms alone but with notable effectiveness when they are combined with known carcinogens.

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THE MONOGRAPHS

INFECTION WITH SCHISTOSOMES
(*Schistosoma haematobium, Schistosoma mansoni* and *Schistosoma japonicum*)

1. Exposure Data

1.1 Structure and biology of schistosomes

1.1.1 *Taxonomy*

Schistosomes are trematode worms ('flukes') belonging to the phylum Platyhelminthes. The adult worms live in the vascular system of birds and mammals ('blood flukes'). Other pathologically important Platyhelminthes include the digenetic trematodes *Opisthorchis*, *Clonorchis*, *Paragonimus*, *Fasciolopsis* and *Fasciola* and the cestodes (tapeworms).

All the schistosomes that mature in man belong to the genus *Schistosoma* of the family Schistosomatidae, which contains 11 other genera, some of which cause cercarial dermatitis (Rollinson & Southgate, 1987). The genus *Schistosoma* contains 19 species (WHO, 1993), five of which (*Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*) are of major pathological importance, while the others are essentially parasites of non-human mammals, although some zoonotic transmission to man does occur. An estimated 600 million people are at risk for schistosomiasis; 200 million are currently infected in 74 countries (WHO, 1993). Probably more than 95% of human infections are due to *S. mansoni* and *S. haematobium*. Several of the 'non-human' species, including *S. mattheei* and *S. bovis*, are of veterinary importance, and both domestic and feral animals are major reservoirs of infection with *S. japonicum* (but not with any of the other species) (Taylor, 1987).

This monograph is restricted to *S. haematobium*, *S. mansoni* and *S. japonicum*.

1.1.2 *Structure*

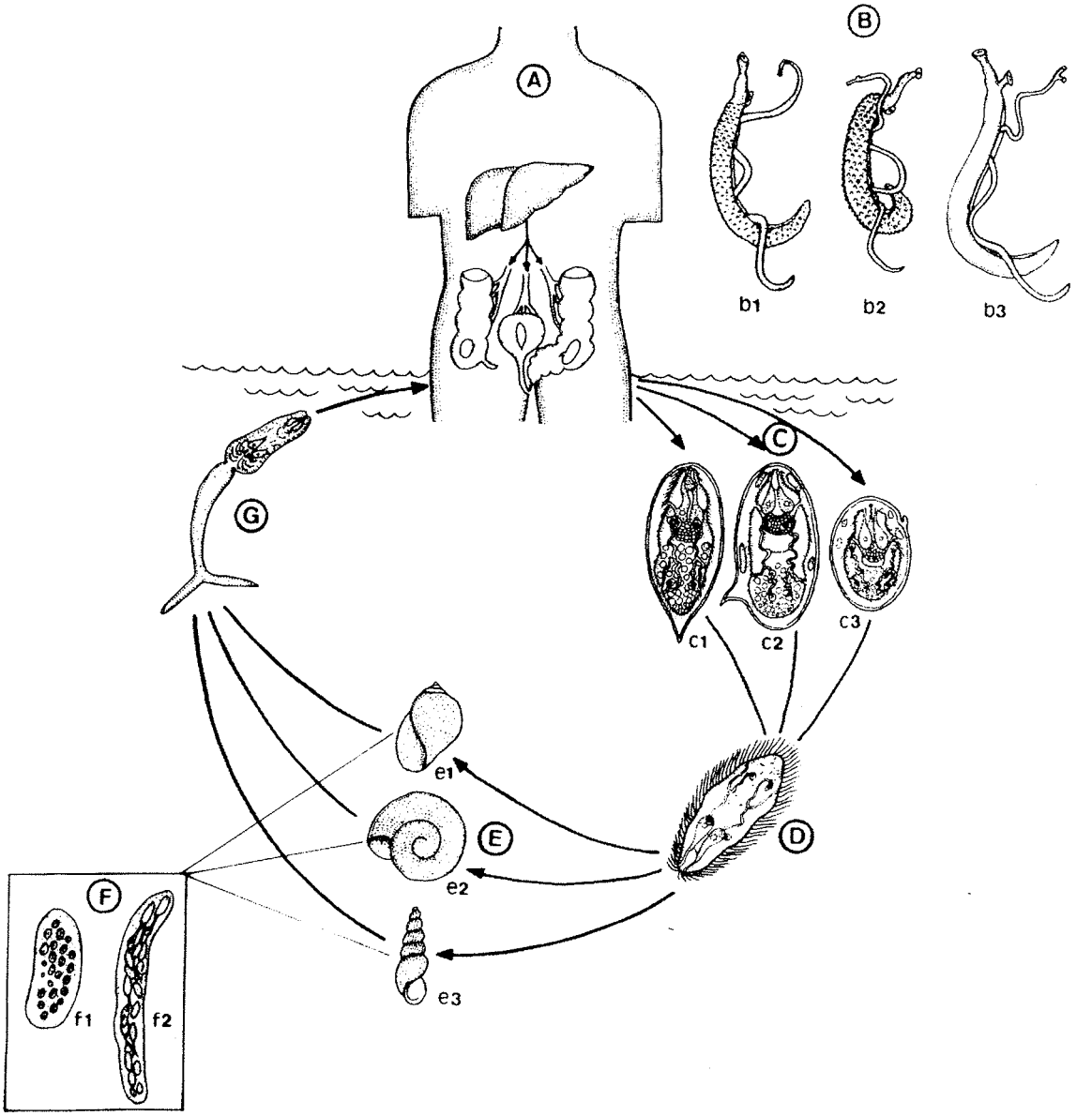
Unlike all other pathologically important trematodes, schistosomes are dioecious (rather than hermaphroditic). The adult worms are about 1 cm long, and the male has a deep ventral groove or schist (hence the term 'schistosome') in which the female worm resides permanently *in copulo*. Worms of each sex have a mouth at the anterior end, which also serves as the anus since there is only one gut opening. Around the mouth is the oral sucker, while nearby, further back, is the ventral sucker. These suckers are much better developed in male worms; they are used mainly for hanging on to the venous epithelium of the host and for locomotion of the worm pair. In order to obtain amino acids for protein synthesis, the adult worms ingest red blood cells and break down the haemoglobin with a haemoglobinase. Small molecules, including glucose, amino acids, purines and pyrimidines, are taken up via transtegumentary absorption; there is evidence that the female derives much of her nutrition

via transtegumentary absorption from the male worm. The metabolism of adult schistosomes is largely anaerobic, by glycolysis (Rumjanek, 1987).

1.1.3 *Life cycle and biology of the adult worm*

Schistosoma do not multiply in the human body. The life cycle of schistosomes is illustrated in Figure 1.

Figure 1. Life cycle of blood flukes



A: definitive host, human; B: adult blood flukes, *Schistosoma haematobium* (b1), *S. mansoni* (b2), *S. japonicum* (b3); C: embryonated egg of *S. haematobium* (c1), *S. mansoni* (c2), *S. japonicum* (c3); D: miracidium; E: intermediate host, *Bulinus* sp. (e1), *Biomphalaria* sp. (e2), *Oncomelania* sp. (e3); F: intramolluscan stages, mother sporocyst (f1), daughter sporocyst (f2); G: cercaria

Adult worms are found either in the vesical plexus of the urinary bladder (*S. haematobium*) or in the mesenteric veins (other species). Adult worms live for up to 30 years (von Lichtenberg, 1987), with a mean lifespan of 3–6 years (Anderson, 1987). They produce large numbers of eggs: 300 per day per female *S. mansoni* and *S. haematobium* and 10 times as many per female *S. japonicum*. About one-half of the eggs transit to the lumen of the urinary bladder (*S. haematobium*) or the intestine (other species), from where they leave the body in the urine or faeces, respectively. A substantial number of eggs are retained in the tissues, where they survive for a further three weeks; these are responsible for inducing most of the pathological manifestations of disease (Warren, 1978).

The eggs are large (e.g. 144 × 58 μm for *S. haematobium*) and consist of an egg shell of tanned protein containing, when laid, about 40 yolk cells and the oocyte. After about one week in the tissues, the mature egg contains the large (150 × 70 μm) ciliated miracidium larva (von Lichtenberg, 1987). It is this life-cycle stage that infects the snail host. Thus, embryonated eggs excreted from the body in urine or faeces and deposited in water hatch to liberate the free-swimming miracidium larvae. If the miracidia can locate an appropriate snail host within a few hours, they penetrate it; if not, they die, as they do not feed.

Within the tissues of the snail, the miracidium is transformed into the mother sporocyst, within which are formed several hundred daughter sporocysts. These migrate from the site of penetration to the digestive gland and reproductive tract of the snail, in which they proliferate internally to produce cercariae, the stage that infects man. This process takes about one month, and from one miracidium several million genetically identical cercariae may be produced by this asexual process during the lifetime of the infected snail.

The cercariae are shed from the snail in response to temperature and light and aggregate at the surface of the water, ready to infect the definitive human host. They swim tail first, locate the host by a combination of chance and chemotaxis and adhere to the skin by their suckers. A cercaria is approximately 0.5 mm long and consists of a head-end, bearing the oral and ventral suckers, and a tail with a pronounced fork. Cercariae respire aerobically, using glycogen as a substrate, but do not feed; therefore, if they do not penetrate the final host within a few hours they die. Cercariae penetrate intact skin rapidly, using proteolytic enzymes produced by the paired penetration glands at their anterior ends; the tail is discarded in the water. Once they are within the skin, a profound metamorphosis takes place, and the cercaria is transformed into the 'skin-stage schistosomulum'. Metamorphosis includes shedding of the cercarial glycocalyx, transformation from the single-lipid bilayer tegument of the cercaria into the double-lipid bilayer of the schistosomulum and various physiological changes, such as a change from aerobic to anaerobic respiration and the acquisition of host molecules, particularly lipids, some of which are incorporated into the tegument (Wilson, 1987).

The schistosomulum then penetrates the basement membrane of the epidermis, using proteinases secreted by the residual penetration glands of the cercaria stage. In mice, this process takes about three days, after which time the schistosomulum enters a lymphatic vessel or capillary in the dermis and is carried passively to the lungs via the right side of the heart (Wilson, 1987). The young schistosomula embolize in the capillaries—being too large to pass through the pulmonary veins—whereupon they again metamorphose, this time to the 'lung-stage schistosomulum', which, unlike the skin stage, is capable of stretching out its body to become long and thin and can cross the capillary bed of the lungs, taking three to six days

to reach the left side of the heart (Wilson, 1987). Schistosomula are then distributed all over the body via the left ventricle, in proportion to cardiac output. Those that embolize in various capillary beds migrate through these to regain the heart and recirculate until they reach the hepatic portal system, a process usually completed within three recirculations. When the hepatic portal system is reached, a third metamorphosis takes place: the elongated migratory forms return to the squat shape of the skin-stage schistosomulum. Blood feeding begins and this is followed by growth, organogenesis and sexual maturation. The mature worms pair up in the intrahepatic portal venules from about four weeks onwards and then migrate to the final sites of oviposition in the vesical plexus (*S. haematobium*) or in the mesenteric veins (all other species).

1.2 Methods for detection of infection

1.2.1 *History taking*

Information derived from simple questionnaires eliciting a history of haematuria is sufficiently accurate to identify nearly all heavily infected people (Mott *et al.*, 1985), and such questionnaires can be used for rapid identification of communities with a high prevalence of *S. haematobium* infection (Lengeler *et al.*, 1991a,b). Validation of the use of questionnaires on history of *S. mansoni* infection for identifying infected people showed a specificity of about 60% (Barreto, 1993). In community-based epidemiological studies of *S. japonicum*, although symptoms of weakness, colicky abdominal pain and diarrhoea were observed in a greater proportion of infected than uninfected individuals, these were not specific to infection (Olveda *et al.*, 1983).

1.2.2 *Clinical diagnosis*

Macroscopic and microscopic haematuria are highly sensitive, specific signs of *S. haematobium* infection in most endemic areas of Africa and the eastern Mediterranean (Savioli & Mott, 1989; Savioli *et al.*, 1990; Eltoum *et al.*, 1992; Lengeler *et al.*, 1993). Testing with chemical reagent strips to detect microscopic haematuria consistently results in the identification of 80% or more of people excreting *S. haematobium* eggs, and gross haematuria is associated with urinary egg counts greater than 50 per 10 ml of urine (Savioli *et al.*, 1990).

Schistosomiasis is a protean, multisystem disease, and the clinical signs and symptoms are often nonspecific (Abdel-Wahab & Mahmoud, 1987; von Lichtenberg, 1987; Olveda & Domingo, 1987; Prata, 1987; Wilkins & Gilles, 1987; Chen & Mott, 1989). Thus, multiple abdominal symptoms may be found in patients infected with *S. mansoni* and *S. japonicum*, of which only a history of bloody diarrhoea is significantly associated with heavy infection (Sleigh & Mott, 1986; Olveda & Domingo, 1987). Schistosome eggs and associated granulomas and fibrosis are frequently detected by liver biopsy. The degree of periportal fibrosis can now be assessed accurately by ultrasonography of the liver (*S. mansoni*, *S. japonicum*) or urinary tract (*S. haematobium*), the latter having replaced intravenous pyelography which was formerly the standard method of assessment (Hatz *et al.*, 1992a,b,c,d; Jenkins & Hatz, 1992; Wei-min *et al.*, 1992).

1.2.3 *Parasitological tests*

The best method for diagnosing infection with mature, egg-producing adult worms is to demonstrate the presence of eggs in the urine (*S. haematobium*) or faeces (other species). In routine medical practice, diagnosis is usually qualitative rather than quantitative. In both techniques, some form of concentration is used to increase sensitivity. Thus, urine samples may be centrifuged or filtered to concentrate the eggs, while eggs in faecal samples are frequently concentrated by the formol–ether technique.

For most epidemiological purposes, however, eggs are counted, although the sensitivity is limited owing to small sample size (de Vlas & Gryseels, 1992; de Vlas *et al.*, 1993). The quantitative relationship between the presence of viable adult worms and faecal or urinary egg counts has been established experimentally (Cheever, 1969) and in autopsy studies (Edington *et al.*, 1970; Smith & Christie, 1986).

For *S. haematobium* infections, filtration through standard filter paper, cellulose membranes, polycarbonate or nylon in filter holders attached to a syringe is a standard quantitative technique. The Kato technique for examination of faeces for the eggs of other *Schistosoma* involves use of a glycerine-impregnated cellophane coverslip over a measured volume of stool, ranging from 10 to 50 mg.

Light and heavy infections can be distinguished reliably by the available faecal and urinary examination techniques in all endemic areas. The limitations of their sensitivity have been well described (Savioli *et al.*, 1990; de Vlas & Gryseels, 1992; de Vlas *et al.*, 1993). A single filtration of a random 10-ml urine sample allows detection of all infected individuals with more than 50 eggs/10 ml of urine (Savioli *et al.*, 1990). Although several quantitative techniques are available for faecal egg counting, their sensitivity is dependent on the amount of stool examined, and the Kato technique has become the standard, allowing comparison of the results of epidemiological studies. A single Kato thick smear allows detection of all people with more than 100 eggs/g of faeces (Barreto *et al.*, 1990; Feldmeier & Poggensee, 1993).

In people with chronic or light infections, eggs may be difficult to demonstrate with these techniques. In such cases, rectal biopsy is sometimes used, followed by microscopic examination of compressed mucosal specimens for eggs. The sensitivity of rectal biopsy is unknown; however, it appears to be highly sensitive clinically, even if the viability of the infection cannot be determined. Sometimes eggs (or adult worms) are found by histopathological examination of lesions taken by biopsy from other anatomical sites or in cytological smears. *S. haematobium* eggs are frequently reported in diverse parts of the urogenital system, and 'ectopic' lesions of the central nervous system caused by *S. japonicum* or *S. mansoni* are seen (Chen & Mott, 1989).

1.2.4 *Immunological tests*

In the past, immediate hypersensitivy-based intradermal tests for *S. mansoni* and *S. japonicum* were widely used in epidemiological studies, but they have been rarely used since 1970 because of the lack of correlation with active infection and the availability of improved parasitological techniques. Using *S. mansoni* adult worm antigens, the sensitivity ranged from 82 to 100% and the specificity from 96 to 99% (Mott & Dixon, 1982); with

S. japonicum adult antigens, the sensitivity ranged from 77 to 99% and the specificity from 95 to 99% (Mott *et al.*, 1987). The age distribution of intradermal reactivity is not known. The specificity is not influenced by other intercurrent infections, except for certain trematode infections; the sensitivity is lower in children than in adults, and the sensitivity of the test and the intensity of the hypersensitivity reaction are greater in infections of long duration. Reactivity persists for years after a successful treatment (Kagan & Pellegrino, 1961).

Researchers have concentrated on *S. mansoni* and *S. japonicum* infections because of the ease with which the parasites can be maintained in the laboratory. Many immunodiagnostic techniques have been described and used experimentally, but so far none has been used consistently or validated in epidemiological studies (Mott & Dixon, 1982; Mott *et al.*, 1987). Difficulty in maintaining *S. haematobium* in the laboratory has limited research in immunodiagnosis of urinary schistosomes (Xue *et al.*, 1993).

Antibody detection assays are very sensitive; however, in epidemiological studies, a positive serological result may be due to either a light infection or the presence of residual antibody from a resolved infection. This is a particular disadvantage now that large-scale chemotherapy campaigns are so frequently carried out (Bergquist, 1992). Antigen detection assays may solve these problems. Several systems are being developed, the most advanced of which involve an enzyme-linked immunosorbent assay with monoclonal antibodies to detect circulating antigens of *S. mansoni* (de Jonge, 1992).

1.2.5 *Establishment of absence of infection*

The absence of infection cannot be established unequivocally. The variation in sensitivity of the diagnostic techniques is such that a combination of diagnostic tests is appropriate to establish absence of infection (Feldmeier & Poggensee, 1993). In the field, at least three successive urine filtration examinations are required to establish the absence of infection with *S. haematobium* (Savioli *et al.*, 1990). For *S. mansoni* infection, five consecutive Kato examinations are required (Barreto *et al.*, 1978). If available, antigen detection assays can be used (see section 1.2.4).

1.3 Epidemiology of infection

1.3.1 *Geographical distribution* (see Table 1 and Figures 2 and 3)

It has been estimated that over 600 million people in 74 countries are exposed to the risk of schistosomal infection, and 200 million are currently infected (WHO, 1993). Schistosomiasis may be the second most important parasitic disease in man after malaria. About 95% of cases are due to *S. mansoni* and *S. haematobium* infections and the remainder to *S. japonicum*, *S. intercalatum* and *S. mekongi*. The geographical distribution of the schistosomes roughly corresponds to the distribution of susceptible snail hosts, which are present in many tropical and subtropical regions. *S. mansoni* is the most widespread species, being prevalent in 52 countries in Africa, the eastern Mediterranean, South America and the Caribbean. *S. haematobium* and *S. mansoni* have a similar distribution in Africa and the eastern Mediterranean; *S. haematobium* does not occur in the Americas. There is a small focus of *S. haematobium* in India, but neither *S. mansoni* nor *S. haematobium* occurs in

Table 1. Geographical distribution of schistosomiasis by species

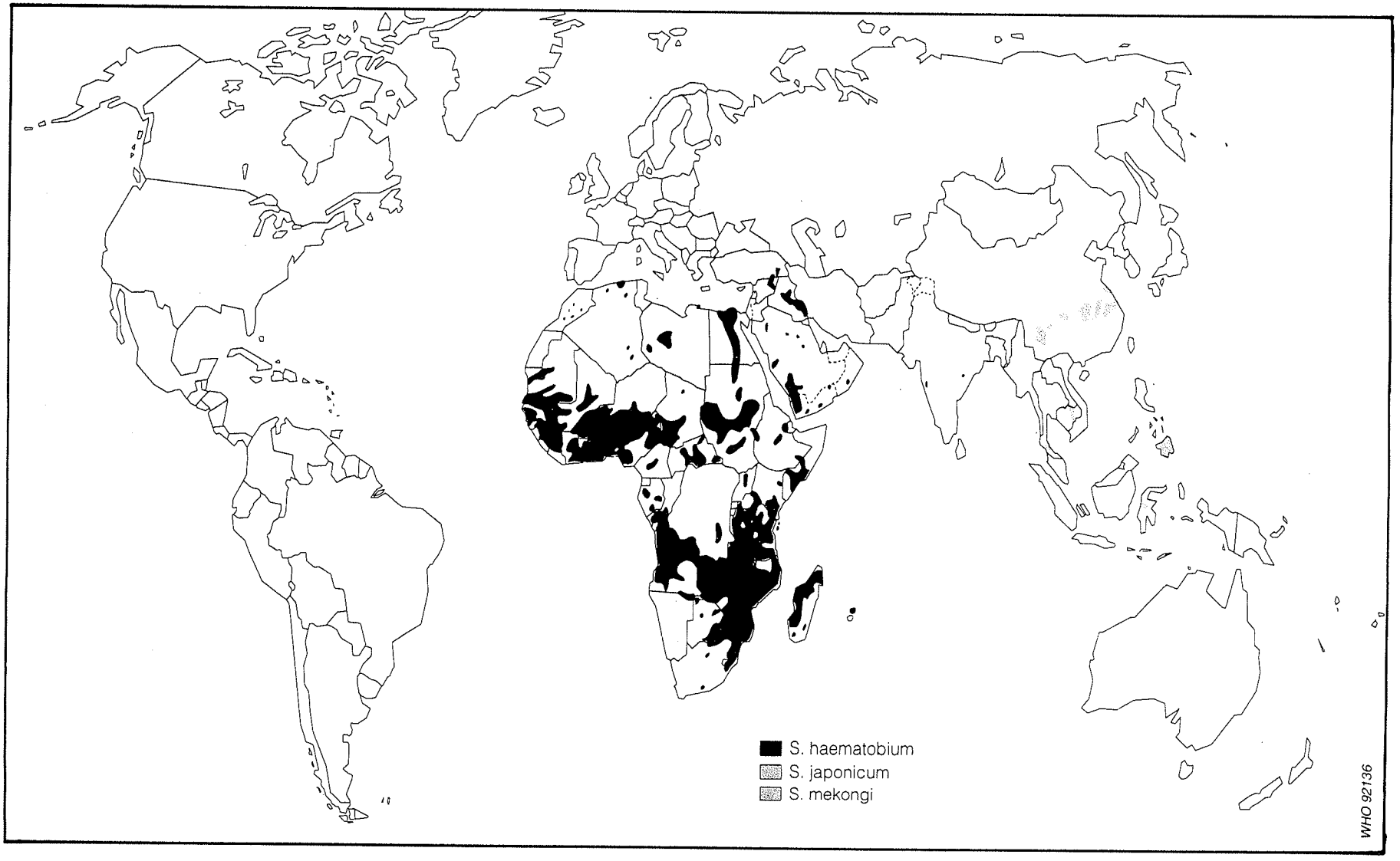
Country or area (by WHO region)	*S. haematobium*	*S. mansoni*	*S. intercalatum*
African Region			
Algeria	+		
Angola	+	+	
Benin	+	+	
Botswana	+	+	
Burkina Faso	+	+	
Burundi		+	
Cameroon	+	+	+
Central African Republic	+	+	+[a]
Chad	+	+	+[a]
Congo	+	+	+[a]
Côte d'Ivoire	+	+	
Equatorial Guinea			+
Ethiopia	+	+	
Gabon	+	+	+
Gambia	+	+	
Ghana	+	+	
Guinea	+	+	
Guinea-Bissau	+	+	
Kenya	+	+	
Liberia	+	+	
Madagascar	+	+	
Malawi	+	+	
Mali	+	+	+[a]
Mauritania	+		
Mauritius	+		
Mozambique	+	+	
Namibia	+	+	
Niger	+	+	
Nigeria	+	+	+[a]
Rwanda	+		
Sao Tome and Principe	+[a]		+
Senegal	+	+	
Sierra Leone	+	+	
South Africa	+	+	
Swaziland	+	+	
Togo	+	+	
Uganda	+	+	
United Republic of Tanzania	+	+	
Zaire	+	+	+
Zambia	+	+	
Zimbabwe	+	+	

Table 1 (contd)

Country or area (by WHO region)	*S. haematobium*	*S. mansoni*	*S. intercalatum*
Region of the Americas			
Antigua		+	
Brazil		+	
Dominican Republic		+	
Guadeloupe		+	
Martinique		+	
Puerto Rico		+	
Saint Lucia		+	
Suriname		+	
Venezuela		+	
Eastern Mediterranean Region			
Egypt	+	+	
Iran, Islamic Republic of	+		
Iraq	+		
Jordan	+		
Lebanon	+		
Libyan Arab Jamahiriya	+	+	
Morocco	+		
Oman	+	+	
Saudi Arabia	+	+	
Somalia	+	+	
Sudan	+	+	
Syrian Arab Republic	+		
Tunisia[b]	+		
Yemen	+	+	
European Region			
Turkey	+		
South-East Asia Region			
India	+		
Indonesia		*S. japonicum*	
Thailand		*S. japonicum*	
Western Pacific Region			
Cambodia		*S. mekongi*	
China		*S. japonicum*	
Japan[b]		*S. japonicum*	
Lao People's Democratic Republic		*S. mekongi*	
Malaysia		*S. malayensis*	
Philippines		*S. japonicum*	

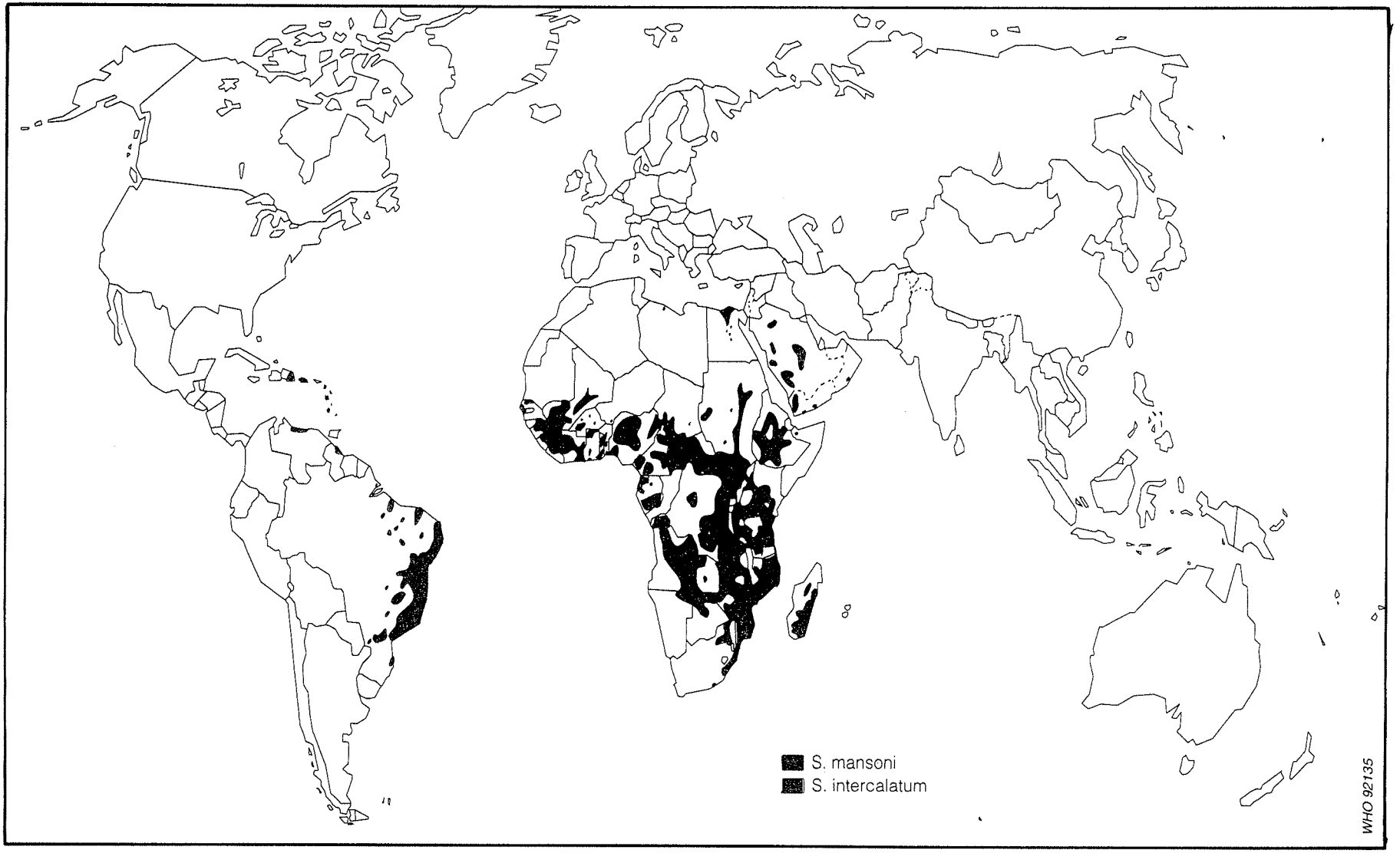
From WHO (1993)
[a]Confirmation required
[b]No recent transmission: Japan, Tunisia

Figure 2. Global distribution of schistosomiasis due to *Schistosoma haematobium*, *S. japonicum* and *S. mekongi*



From WHO (1993)

Figure 3. Global distribution of schistosomiasis due to *Schistosoma mansoni* and *S. intercalatum*



From WHO (1993)

central or east Asia; *S. japonicum* is endemic in three countries (China, the Philippines and Indonesia), while the related *S. mekongi* is restricted to the Mekong River basin in the Lao People's Democratic Republic and Cambodia. In Africa, *S. mansoni* and *S. haematobium* often coexist, and mixed infections are common. *S. intercalatum*, a much rarer species than *S. mansoni* or *S. haematobium*, is restricted to foci in 10 central and West African countries.

1.3.2 *Risk factors for infection*

Contact with contaminated freshwater is the major risk factor of infection (Jordan & Webbe, 1993). Many other environmental factors influence the distribution, prevalence, intensity of infection, morbidity and mortality of schistosomiasis (WHO, 1993). Among these are the type and size of intermediate snail host populations, human population density and behaviour in relation to freshwater bodies, and climatic and hydrological features. Infection may be constant in endemic areas owing to repeated contact with water, particularly among children.

Susceptibility to infection is influenced by genetic factors (Abel *et al.*, 1991), but genetic differences between parasites are not known to influence their infectivity. Acquisition of infection depends on the duration of exposure, proportion of the body surface exposed, degree of body movement during exposure, presence of intermediate snail hosts, cercarial concentration in the water and water temperature. These conditions are fulfilled in endemic areas, usually in open water bodies where frequent recreational contact occurs.

Since schistosomes, like most other helminths, do not multiply in man, it is a striking feature of schistosome epidemiology that, although the prevalence of infection may be very high, significant symptoms are present in only the small segment of people who are most heavily infected. The decline in prevalence and intensity of infection after the second decade of life is believed to be due mainly to the gradual acquisition of immunity, although other age-related factors, such as decreasing contact with infected water and physiological changes associated with the onset of puberty, may also be important (Hagan *et al.*, 1991; Rihet *et al.*, 1991; Dessein *et al.*, 1992; Dunne *et al.*, 1992) (see Figures 4 and 5).

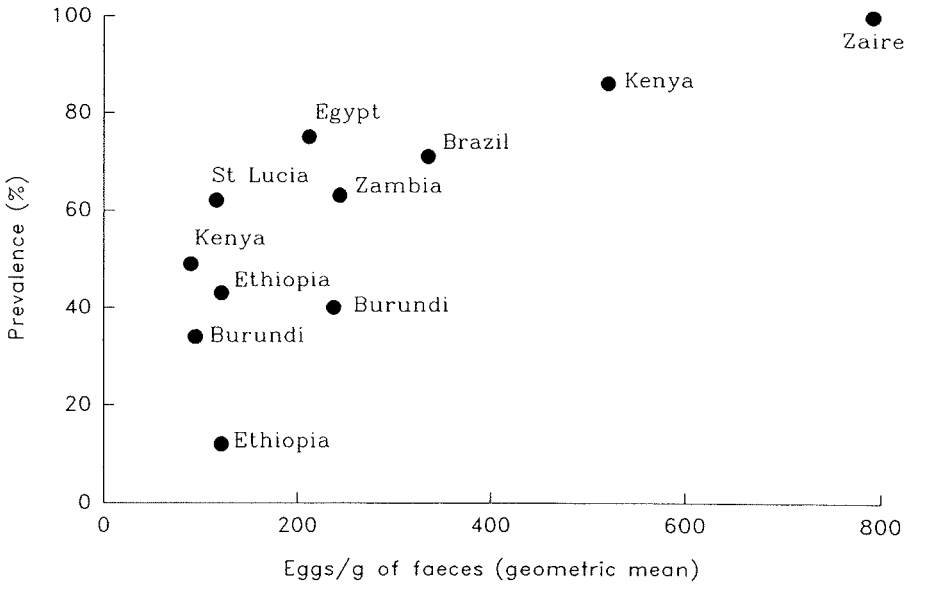
1.3.3 *Aggregation of infection*

Within any endemic area, transmission is highly focal and the prevalence and intensity of infection vary between households, communities and progressively more population agglomerations. This heterogeneity or aggregation is determined by the risk factors cited in section 1.3.2. In common with other worms, *Schistosoma* are not randomly distributed among infected persons but are aggregated in heavily infected people in a manner best described by a negative binomial distribution. The amount of tissue damage caused by the *Schistosoma* infection is roughly proportional to the numbers of worms present; it is the heavily infected segment of the population that is at greatest risk of developing disease and which contributes the most to transmission of the parasite.

1.3.4 *Prevalence and intensity of infection*

For epidemiological studies, the intensity of infection is measured by the number of eggs/10 ml of a urine sample (*S. haematobium*) or per gram of faeces (all other species).

Figure 4. Relationship between overall prevalence and intensity of infection with *Schistosoma mansoni* as determined by the Kato technique in different endemic areas in various studies



From Jordan & Webbe (1993)

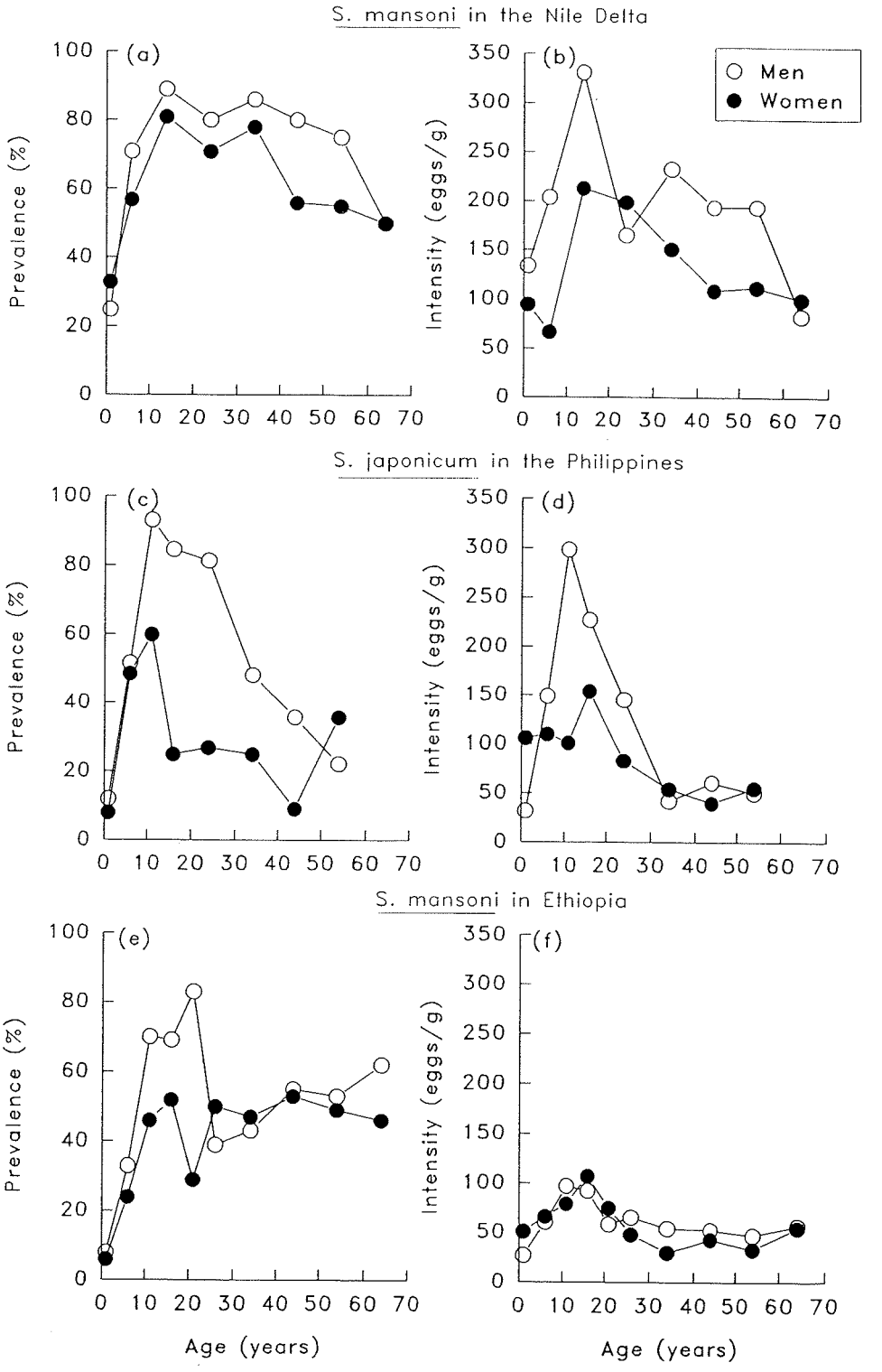
Definitions of 'heavy' infections are routinely included in most epidemiological studies (Sleigh & Mott, 1986). Throughout areas endemic for urinary schistosomiasis, most infection in people who excrete more than 50 eggs/10 ml of urine is associated with haematuria (Mott *et al.*, 1983). The definition of heavy infection due to *S. mansoni* varies from a mean of 16 eggs/g of faeces in areas of low prevalence such as Puerto Rico (Hiatt *et al.*, 1980) to 1000 eggs/g of faeces in Burundi (de Vlas & Gryseels, 1992). About 10% of infected people in areas endemic for *S. mansoni* have heavy infections. *S. japonicum* infections have been considered to be heavy when more than 400 eggs/g of faeces are found; they occur in up to 4% of some populations (Olveda & Domingo, 1987).

Analysis of 11 methodologically similar studies (Jordan & Webbe, 1993) showed that there is a general trend to a proportional relationship, i.e. the higher the prevalence, the higher the intensity (Figure 4). A similar relationship was seen for *S. haematobium* infection, but few similar population-based studies have been reported using comparable methods.

The peak prevalence of all *Schistosoma* infections occurs in the second decade of life. In general, the decrease in intensity of *S. haematobium* infection after that time is accompanied by a comparatively greater decrease in prevalence than in *S. mansoni* infection. That is, while the intensity of *S. mansoni* infection tends to decrease in the same period, the prevalence remains high, i.e. a few eggs are excreted over a long period.

Few studies have been carried out on the interaction of *S. mansoni* and *S. haematobium* infections. The data reported by Robert *et al.* (1989) suggested that the intensity of *S. haematobium* in mixed infections was greater than that in infections with *S. haematobium* alone.

Figure 5. Age–prevalence patterns based on faecal and urinary egg counts



(a) and (b) from Abdel-Wahab *et al.* (1980); (c) and (d) from Hiatt (1976); (e) and (f) from Olveda *et al.* (1983). Intensities are geometric means.

1.3.5 *Sex-related patterns of infection*

Differences in the sex distribution of infection were seen in three selected epidemiological studies (Figures 4 and 5). In general, although not universally, the prevalence and intensity of infection are higher in men than in women, owing to greater employment in agricultural work. The interpretation of any statement about sex differences must, however, take into account the focality of infection and its variable distribution (see section 1.3.3). In predominantly Islamic countries such as Egypt, the prevalence and intensity of urinary schistosomiasis tend to be lower in girls and women than in boys and men (El-Malatawy *et al.*, 1992) owing to lower rates of contact with water.

1.3.6 *Relationship of morbidity to intensity of infection*

Morbidity due to *Schistosoma* infection becomes apparent during the period of peak prevalence and intensity of infection as well as many years later. In urinary schistosomiasis due to *S. haematobium*, the intensity of infection is correlated with morbidity, especially in children. The degree of haematuria and proteinuria detectable by chemical reagent strips is correlated with the intensity of infection (Mott *et al.*, 1983; Savioli *et al.*, 1990). Changes in the urinary bladder and ureters detected radiologically (Forsyth, 1969; Pugh *et al.*, 1979; Warren *et al.*, 1979) or by ultrasound (Hatz *et al.*, 1992a), cystoscopic abnormalities of the urinary bladder (Abdel Salam & Ehsan, 1978) and pathological signs at autopsy (see section 4.1) are also correlated with intensity of infection.

Although *S. japonicum* adults lay more eggs per day (see section 1.1.3), the rates of hepatic and splenic enlargement are similar to those observed in *S. mansoni* infections when the egg counts are similar (Olveda & Domingo, 1987).

Kloetzel (1964) showed in population-based studies in Northeast Brazil that the rates of splenomegaly associated with *S. mansoni* infection are proportional to the intensity of infection, as measured by faecal egg counts, particularly in the first two decades of life.

1.3.7 *Relationship of morbidity to mortality from infection*

Annual mortality due to *S. haematobium* infection in East Africa has been estimated at 2/1000 infected adults (Forsyth, 1969). The proportional contribution of urinary bladder cancer or hydronephrosis leading to end-stage renal disease is not known.

In 1984, annual mortality due to portal hypertension caused by schistosomiasis from *S. mansoni* in Brazil was estimated at 0.5/100 000 total population; at the same time in Suriname, the figure was estimated to be 2.4/100 000 inhabitants. The control of schistosomiasis through large-scale chemotherapy in Brazil was associated with a decline in related annual mortality between 1977 and 1988 from 0.67 to 0.44 deaths per 100 000 inhabitants (WHO, 1993).

Before the introduction of praziquantel in China, severe acute schistosomiasis due to *S. japonicum* resulted in a mortality rate of 2.5–20.7%. Mortality from schistosomiasis during 1975–79 in 10 counties in the Jiaxing area, Zhejiang Province in China was reported to vary from 0.69 to 39.8/100 000 in men [median, 15.1] and from 0.45 to 44.6/100 000 in women [median, 7.7] (Liu *et al.*, 1983). Cumulative (0–64) mortality rates during 1973–75 were reported from 49 counties in various Chinese provinces. No mortality was seen among men

in 29 counties or among women in 36 counties; the rates in counties with some deaths from schistosomiasis varied from 0.03 to 37.2/1000 for men [median, 1.3/1000] and 0.07–42.1/1000 for women [median, 1.4/1000] (Chen *et al.*, 1990). In Leyte, the Philippines, annual mortality among 135 untreated patients was 1.8% (Blas *et al.*, 1986). More widespread use of antischistosomal drugs in highly endemic areas should reduce both morbidity and mortality.

1.4 Clinical disease in humans (other than cancer)

Infection with *Schistosoma* is not synonymous with clinical disease: many infections are asymptomatic. The clinical outcome of schistosomal infection is affected by many factors, including: the target organs of the different species of *Schistosoma*; the intensity and duration of infection; host HLA type and race (Salam *et al.*, 1979; Sasazuki *et al.*, 1980; Kamel *et al.*, 1984; Kojima *et al.*, 1984; Wishahi *et al.*, 1989; Ohta *et al.*, 1990; Abel *et al.*, 1991; Hafez *et al.*, 1991; Proietti *et al.*, 1992); host immunological responses (Phillips & Lammie, 1986; Boros, 1989; Weinstock, 1992); and concomitant infections, notably with hepatitis viruses (Bassily *et al.*, 1992; Uemura *et al.*, 1992; Chen *et al.*, 1993; Darwish *et al.*, 1993). Therefore the manifestations of schistosomiasis vary greatly from patient to patient and among endemic areas.

Most of the pathological manifestations of schistosomal infections are due to fibrosis consequent to immunological reactions to parasite eggs embolized in tissues (Abdel-Wahab & Mahmoud, 1987; von Lichtenberg, 1987; Prata, 1987; Wilkins & Gilles, 1987; Chen & Mott, 1989). As adult *S. haematobium* worms reside in the vesical plexus and ureteric veins, the most badly affected organs are the urinary bladder and ureters, where egg deposition is heaviest. The other schistosome species live in the mesenteric veins, depositing their eggs in the intestine and liver.

The larval forms of the schistosomes are also involved in the disease process. Repeated penetrations of the skin by cercariae (particularly of non-human species of schistosomes, which die in the epidermis) can cause a severe form of dermatitis, which is known to be a complex, immunologically mediated reaction involving both immediate and delayed hypersensitivity components (Boros, 1989).

The presence of maturing schistosome infections with *S. mansoni* or *S. japonicum* can cause an acute febrile illness called 'Katayama syndrome' or 'acute schistosomiasis'. Although the exact timing of exposure to cercariae is usually difficult to establish, in most cases the onset of this syndrome appears to coincide with the start of egg laying by adult worms, three to four weeks after exposure to cercariae (eggs do not appear in the faeces for at least one week more). Since the symptoms of acute schistosomiasis resemble those of serum sickness, the former may also be a form of type III immune complex disease (Butterworth, 1993). The cercarial glycocalyx contains carbohydrate antigens which cross-react with antigens of the egg stage, and small soluble immune complexes may be formed in the period of initial egg laying when egg antigens are present in greater amounts than low-affinity antibody. As antibody titre and affinity increase, larger insoluble immune complexes are phagocytosed and the symptoms subside. Alternatively, treatment of the worms leads to resolution by removal of the source of antigen.

Mature *S. haematobium* lay their eggs in the subepithelial tissues of the urinary bladder and ureters. Those eggs that leave the body via the urine cause petechial haemorrhages which, when sufficiently numerous, result in visible haematuria. The aggregation of large numbers of eggs and granuloma formation in the tissues of the urinary bladder and ureters can lead to filling defects in the urinary bladder and stenosis and eventual obstruction of the ureters. Eventually, inflammatory polyps may subside, leaving fibrous 'sandy patches' on the urothelium. Eggs retained in the subepithelial tissues have a life span of three weeks; they then 'mineralize', acquiring calcium and magnesium salts, and subsequently persist for many years as 'calcified' black eggs. If these are very numerous, they form a ring of radio-opaque tissue that is clearly visible on an X-ray photograph of the so-called 'calcified bladder'. The progressive accumulation of eggs and the attendant inflammatory and granulomatous host reactions usually affect urinary bladder function, and frequency of micturition and dysuria are common symptoms. Obstruction of urine flow in the ureters causes hydroureter and hydronephrosis, and failure of the ureteric sphincter can lead to ascending bacterial infection of the ureters and kidneys (pyelonephritis) (von Lichtenberg, 1987; Wilkins & Gilles, 1987).

Adult *S. haematobium* worms often migrate to the veins of pelvic organs other than the urinary bladder and ureters to produce eggs, with their attendant inflammatory and granulomatous reactions. Dead (calcified) eggs are frequently seen in the submucosa of the colon (although they are rarely excreted in the faeces), where they are of little pathological consequence. More important are the reactions to eggs in the tissues of the reproductive tract: ectopic schistosomiasis of the vagina, uterus, fallopian tubes and ovaries can result in sterilization or misdiagnosis as cancer (Berry, 1966; El-Maraghy *et al.*, 1982). Similarly, schistosomal orchitis can be mistaken for malignancy (Mikhail *et al.*, 1988). Many eggs that fail to lodge in the pelvic organs are shunted to the lungs, where they cause granulomatous reactions. Central nervous system involvement is, perhaps surprisingly, rare in *S. haematobium* infection.

Mature worms of the other species deposit their eggs in the distal mesenteric veins in the submucosa of the intestine. About one-half of these eggs transit the bowel and leave the body via the faeces, causing, as they do so, petechial haemorrhages which often give rise to visible traces of blood in the faeces. Large clusters of eggs in the mucosa can cause the formation of haemorrhagic polyps and colitis, with resulting serious blood loss and colonic dysfunction (El-Masry *et al.*, 1986; Mohamed *et al.*, 1990).

Many of the eggs fail to lodge in the submucosa and are swept upstream to the intrahepatic branches of the hepatic portal vein. Being too large (approximately 45 μm in diameter) to enter the sinusoids, they embolize and elicit granulomatous reactions. Large granulomas are formed in sensitized individuals, which are 100 times the volume of the eggs themselves. The granulomas consist of a complex, mixed population of cell types, mostly lymphocytes, monocytes, macrophages, eosinophils, epithelioid cells and fibroblasts. Collagen deposition occurs in granulomas in response to cytokines produced by granuloma lymphocytes. When the miracidium dies (after three weeks), further fibrosis ('scar tissue') may occur, although the granulomas are sometimes resorbed completely. The gradual accumulation of granulomas in liver tissue can cause hepatomegaly and portal hypertension. Fibrosis occurs not only within the periovular granulomas but also at distant sites, around large branches of the intrahepatic portal vein, probably in response to cytokine action. In

prolonged infections, significant periportal fibrosis (Symmers' fibrosis) often develops, associated with severe portal hypertension, development of gastrooesophageal varices and haematemesis. Splenomegaly is present, caused partly by congestion and partly by a reactive hyperplasia (Abdel-Wahab & Mahmoud, 1987; von Lichtenberg, 1987; Prata, 1987).

Chronic *S. mansoni* and *S. japonicum* infections are usually well tolerated by the patients for many years because the liver lesions are restricted to the portal triads and hepatocytes function normally. The development of fibrosis and collateral circulation may, however, progress insidiously, and fatal haematemesis may occur without warning. Some patients develop liver failure, perhaps caused by concomitant infection with hepatitis viruses (Chen *et al.*, 1993).

If collateral circulation is present, many eggs bypass the liver and instead embolize in the lungs (El-Rooby, 1985), where progressive accumulation, granuloma formation and fibrosis develop, leading to pulmonary arteritis and cor pulmonale (right ventricular hypertrophy). The development of collateral circulation also predisposes to an immune complex-mediated glomerulonephritis (Andrade & Van Marck, 1984).

S. mansoni and *S. japonicum*, but rarely *S. haematobium*, sometimes reach the central nervous system and cause transverse myelitis. *S. japonicum* eggs tend to localize in the brain and may be associated with epilepsy (Norfray *et al.*, 1978; El-Rooby, 1985; Scrimgeour & Gajdusek, 1985).

Particularly when infection intensity is high, schistomiasis can lead to decreased working capacity (Parker, 1992, 1993), and there is increasing evidence that *S. japonicum* (McGarvey *et al.*, 1993), *S. haematobium* (Stephenson *et al.*, 1985, 1989) and *S. mansoni* (Jordan & Randall, 1962; de Lima e Costa *et al.*, 1988; Corbett *et al.*, 1992; Stephenson, 1993) can each affect child growth and nutrional status adversely. It has also been shown (Kimura *et al.*, 1992) that *S. haematobium* infection depresses cognitive function in children.

1.5 Treatment and control

1.5.1 *Treatment*

Safe, effective chemotherapy has been available for the past 20 years against all the schistosomes that affect man (WHO, 1993). The most versatile drug is praziquantel, which is effective in a single oral dose against all species of schistosomes (and some other trematodes and cestodes). Large-scale treatment is costly (US$ 0.35 per treatment), and, in areas of infection with *S. haematobium* only, the much cheaper metrifonate may be preferred, which, however, must be given in two or three doses at two-week intervals. Metrifonate is effective only against *S. haematobium*, while the third available drug, oxamniquine, is effective only against *S. mansoni*, for which it provides safe and effective treatment. None of these drugs is significantly effective against infections by immature worms; thus, prophylactic treatment is not available. Katayama syndrome is usually treated symptomatically for hypersensitivity reactions, but praziquantel is also given to kill adult worms as they mature. In advanced or ectopic disease, surgery for anatomical consequences and complications of infection may be necessary, but, even in advanced cases, antischistosomal drug therapy usually produces great improvement.

Treatment of all forms of schistosomiasis with safe, effective antischistosomal drugs (i) results in a high rate of resolution of infection, even in endemic areas where reinfection is a risk; (ii) prevents development of disease in people with heavy infection; (iii) arrests progression of existing severe disease; and (iv) reverses some manifestations of disease, such as haematuria and proteinuria, particularly in children. Liver fibrosis caused by *S. mansoni* and *S. japonicum* infection is usually arrested by the treatment and may even be reversed (Mohamed-Ali *et al.*, 1991; Wei-min *et al.*, 1992). Similarly, in cases of *S. haematobium* infection, hydroureter and hydronephrosis are reversible by treatment (Doehring *et al.*, 1986; Hatz *et al.*, 1990; King *et al.*, 1990).

1.5.2 *Control*

Control of schistosomiasis in the community may in practice by achievable by removing the adult worms by chemotherapy, by eliminating the snail intermediate hosts by modification of their habitat or by chemical attack, by changing human behaviour through health education, by providing safe water supplies and sanitation, so that excreta containing live eggs do not reach water containing snails, and by ensuring that people avoid water contaminated with cercariae.

Effective drugs are available. Trivalent antimonials were introduced in 1918, although these toxic compounds were far from ideal for control programmes since they required repeated intravenous injections. Chemical control of snails by molluscicides became possible in the 1920s, when copper sulfate was introduced for the control of the aquatic vectors of *S. mansoni* and *S. haematobium*, and when lime was first used to attack the amphibious vectors of *S. japonicum*.

Using integrated control measures since the 1920s, the Japanese eventually eradicated schistosomiasis by the end of the 1970s (Kitani & Iuchi, 1990). Similarly, in the much more extensive endemic areas of *S. japonicum* in China, unremitting integrated control measures over a 40-year period have reduced the prevalence of schistosomiasis by 90% (Chen, 1989; Anon., 1992). Eradication has also been achieved in two other countries: *S. haematobium* has been eliminated in Tunisia, and *S. mansoni* in Monserrat (WHO, 1993). In several countries, particularly those where schisosomiasis was identified early on as a major public health problem, such as Brazil, Egypt, the Islamic Republic of Iran, the Philippines and Venezuela, significant reductions in disease prevalence have been achieved, usually by national control programmes that incorporate integrated measures. Even in cases where prevalence of infection has remained high, the prevalence of serious disease manifestations (such as Symmers' fibrosis and fibro-obstructive lesions of the urogenital tract) has often been reduced, largely by the use of population-based chemotherapeutic campaigns (WHO, 1993).

Set against this, however, is the demographic increase in younger people, who are most affected by the disease, thus increasing the size of the susceptible population. This, combined with the expansion of water resource developments and irrigation, has led to spread of the disease to new areas and to intensification of transmission in existing endemic areas. The WHO (1993) report on schistosomiasis control thus concluded that the global number of infected cases was similar to that in 1984. Furthermore, in only a very few areas has the snail vector been eradicated, so that, if control measures break down or are relaxed, the disease will rapidly sweep back and may in fact become worse than before because of loss of

immunity by the population. Currently, no antischistosomal vaccine for humans is available, although intensive efforts are being made to develop one.

2. Studies of Cancer in Humans

Concern about a causal relationship between infection with schistosomes and cancer is based on observations of patients who have been exposed to *S. haematobium*, *S. japonicum* and *S. mansoni*.

2.1 Descriptive studies

2.1.1 *Schistosoma haematobium*

The proportion of all cancers represented by urinary bladder cancer varies greatly within Africa and the Middle East, and the ratio of male to female frequency of occurrence is nearly as variable (Parkin, 1986). In Egypt, the proportion of bladder cancers among all cancers in men is twice that in Zambia, four times that in Zimbabwe and 10 times that in Algeria. Very few formal assessments of the correlation between bladder cancer incidence and the prevalence of *S. haematobium* have been done, but there are many informal descriptions of geographical correspondence between the areas affected by the two diseases.

Most of the early clinical descriptions of urinary bladder cancer in connection with evidence of schistosomiasis come from the Nile Delta, where there are few unexposed populations and no population-based incidence data (see section 2.2.1); however, in countries with less universal exposure, observations have been made on the geographical relationship between exposure to *S. haematobium* and bladder cancer occurrence. The common geographical pattern of occurrence of *S. haematobium* and bladder cancer has been noted by investigators in almost all endemic African countries (Table 2).

In addition to the link between the risk of a subpopulation for a haematobium schistosomiasis and the risk of the same population for urinary bladder cancer, a slightly more direct link has been noted; the proportion of bladder cancers that are squamous histologically in the population of a country is related to the proportion of cancerous bladder specimens from that population which contain evidence of past schistosomal infection in the form of eggs or egg remnants (Lucas, 1982a). This has been noted even within countries; in Iraq, for example, 36.1% of bladder cancer cases from the north are squamous-cell tumours and 4.9% have evidence of *S. haematobium*, whereas in the south, where *S. haematobium* is more prevalent, 54.8% are of the squamous variety and 32.2% have evidence of *S. haematobium*; those from the central part of the country show intermediary rates of 48.5% and 20.7%, respectively (Al-Fouadi & Parkin, 1984).

The two diseases have other characteristics in common. In a description of the pattern of urinary bladder cancer by occupation in the Nile Delta, 99% of the bladder cancers occurring in high-risk male agricultural workers (*fellahin*) were found to be associated with histological evidence of *S. haematobium* infection, whereas only 52% of the cases occurring in men with lower-risk occupations showed such evidence (Makhyoun *et al.*, 1971).

Table 2. Descriptive studies of infection with *Schistosoma haematobium* and urinary bladder cancer

Reference	Location	Outcome index	Exposure index	Geographical correlations	Secular or occupational correlations	Correlated sex ratios or age distributions
Talib (1970)[a]	Iraq, referral hospital	Proportional frequencies	Common knowledge	More patients from south and centre, where *S. haematobium* is endemic	–	–
Anjarwalla (1971)	Kenya, referral pathology service	Proportional frequencies	Frequency of schistosomiasis diagnoses and school surveys	Patients from coastal area, where schistomiasis is common	–	–
Makhyoun *et al.* (1971)[a]	Egypt, Nile Delta University hospital	Proportional frequencies	Common knowledge	–	Cases in male *fellahin*: 99% histologically *S. haematobium* egg-positive Cases in men in other occupations: 52% positive	Exceptionally higher sex ratio for bilharzial cases (11.8:1) than for non-bilharzial (4.8:1), low-risk British (4.1:1) cases or high-risk Mozambican cases with exposure during field work (0.9:1)
Anthony (1974)	Uganda, referral hospital	Proportional frequencies	Frequency of schistosomiasis diagnoses	Bladder cancer, including squamous-cell cancers, unrelated to small foci of schistosomiasis	–	–
Bowry (1975)[a]	Kenya, referral pathology service	Proportional frequencies	Frequency of schistosomiasis diagnoses and school surveys	Cancer foci on coast and near Lake Victoria, both known foci of schistosomiasis	–	–
Malik *et al.* (1975)[a]	Sudan, referral hospital	Proportional frequencies	Ministry of Health records of 'highest endemicity'	Correspondence between frequency of bladder cancer and endemicity by province	–	–
Keen & Fripp (1980)[a]	South Africa (Transvaal)	Frequencies identified in regional surveys	None explicit	–	–	Wide variations in sex ratio (from 2:1 to 1:2) according to region and tribe
Lucas (1982)[a]	Africa	Proportional frequencies	Histological identification of *S. haematobium* eggs in bladder specimens	Geographical distribution of percentage of histologically *S. haematobium* egg-positive tumours correlated directly with percentage of all bladder cancers that are squamous-cell and inversely with the percentage that are transitional-cell tumours		

Table 2 (contd)

Reference	Location	Outcome index	Exposure index	Geographical correlations	Secular or occupational correlations	Correlated sex ratios or age distributions
Hanash (1984)	Saudi Arabia, referral hospital	Proportional frequencies	Known distribution of *S. haematobium* endemicity	Bladder cancer cases commonly come from endemic communities	–	–
Al-Fouadi & Parkin (1984)[a]	Iraq, urban hospitals	Registered cases	'Known distribution of *S. haematobium* endemicity'	Percentage of tumours that are squamous-cell and percentage of tumours that contain histologically identifiable *S. haematobium* eggs closely related to southern latitude [proximity to the river delta]	–	–
Kitinya *et al.* (1986)[a]	United Republic of Tanzania, referral hospital	Proportional frequencies	Known distribution of snail vectors in relation to altitude	Low proportion of squamous-cell tumours and low prevalence of *S. haematobium* at high elevations near Mt Kilimanjaro	–	–
Tawfik (1988)[a]	Egypt, referral hospital	Proportional frequencies	Histological identification of *S. haematobium* eggs in bladder specimens; records of control programme	–	High bladder cancer proportional frequency despite 20 years of successful control efforts (prevalence reduced from 60 to 10% in one province)	High sex ratio correlated with documented intensity of infection. As period of successful control efforts lengthens, mean age of bladder cancer increases.
Thomas *et al.* (1990)	Zimbabwe, referral hospital	Proportional frequencies	National prevalence surveys among schoolchildren	Estimated bladder cancer incidence correlated with prevalence of *S. haematobium* infection ($r = 0.87$; $p < 0.01$). Ratio of squamous-cell to transitional-cell tumours linked to *S. haematobium* prevalence: 12:1 where prevalence was 67%, 2:1 where prevalence was 17%	–	Sex ratio for squamous-cell tumours, 1.0; for transitional-cell tumours, 2.9:1.

[a]Correlation not formally tested

Whereas in the Nile Delta, where men do most of the agricultural work, the ratio of male to female cases of urinary bladder cancer with histological evidence of past infection may be as high as 12:1 (Makhyoun *et al.*, 1971), the sex ratio among those without such evidence approximates the 4:1 ratio seen in the United Kingdom (Prates & Gillman, 1959). In contrast, in Mozambique (Prates, 1963) and adjacent regions of the Transvaal in South Africa (Keen & Fripp, 1980), where women do most of the agricultural labour and are therefore more commonly infected, the sex ratios are reversed to 1:1.1 or even 1:2, even though ratios of 2:1 prevail among cases referred from nearby areas. The sex ratio of bladder cancer cases has also been linked to the histologically measured intensity of infection in tumour specimens, and ranged from 8.7:1 in heavily infected people, to 4:1 in those who are lightly infected, to 2:1 in those without eggs in Egypt (Tawfik, 1988).

In a community in Angola, where both males and females work in agriculture, the minimal age of infection with *S. haematobium* was 11 years. The mean age of patients with urinary bladder carcinomas associated with schstosomiasis was 44 years. The sex ratio was 1.6:1 for bladder carcinoma associated with schistosomiasis and 3.2:1 for bladder carcinoma not associated with schistosomal disease ($p \sim 0.05$) (da Silva Lopes, 1984).

It should be noted, however, that in Uganda, squamous-cell carcinomas of the urinary bladder are commoner than in Europe or North America in the absence of any relationship to known *S. haematobium* prevalence (Anthony, 1974).

Because of the lack of population-based cancer registration, the secular trends in incidence of squamous- or transitional-cell carcinomas of the urinary bladder have not been formally evaluated. In an area of the Nile Delta where the prevalence of *S. haematobium* infection was brought from a level of 60% in 1968 to 10% in 1988, no impact upon the rate of bladder cancer was clinically evident at the end of that period, although the mean age at diagnosis had increased (Tawfik, 1988).

2.1.2 *Schistosoma mansoni*

No description has appeared of the geographical occurrence of cancer in relation to the prevalence of *S. mansoni* infection. In relation to liver cancer, one observer pointed out that the pattern of occurrence in Africa and South America does not correspond to that which would be expected on the basis of a strong association with *S. haematobium* (Edington, 1979). The absence of any geographical relationship between colorectal cancer and colorectal schistosomiasis in Africa is even clearer. Despite wide variations in the geographical distribution of *S. mansoni*, colorectal cancer occurs in Africa with remarkable uniformity, insofar as the proportion of cases among all cancers provides pertinent information (Parkin, 1986). Moreover, reports from multiple centres in north, east, south and west Africa all indicate that evidence of schistosomal infection in colorectal tumour specimens is no commoner than would have been expected on the basis of the known prevalence of infection (Murray, 1967).

2.1.3 *Schistosoma japonicum*

The geographical co-occurrence of *S. japonicum* and cancer has been assessed formally (Table 3). Unfortunately, interpretation of the geographical patterns of occurrence of liver and colorectal cancers in Asia is difficult, because of known variations in the distribution of other causes of the same neoplasms, including hepatitis viral infection, dietary nutrients and carcinogenic dietary contaminants such as aflatoxins. In particular, a large correlation study from China assessed the association between mortality from schistosomiasis and from colorectal, liver, oesophageal and gastric cancers (Liu *et al.*, 1983). Correlations were calculated at two geographical levels: in 24 provinces of varying endemicity and in 10–98 counties within six provinces of high endemicity. [The Working Group noted that, in addition to the problems common to the interpretation of all correlation studies (see Preamble, p. 22), interpretation of studies correlating mortality from cancer and from schistosomiasis are complicated by the low diagnostic specificity of the latter cause of death; however, such misclassification of cause of death would probably lead to an underestimated correlation coefficient.]

(*a*) *Liver cancer*

In the study of Liu *et al.* (1983) in areas of high endemicity in China, significant correlations were found for both men and women in one province, while in four other provinces, the correlations were significantly positive only for women. No correlation was found in an analysis of 24 provinces, or in the seven endemic counties in Jiangsu Province (Guo *et al.*, 1984).

Within areas of Yamanashi Prefecture, Japan, classified on the basis of prevalence rates of schistosomiasis in 1958–62 [survey method not specified], the standardized mortality ratios for liver cancer on the basis of mortality in Japan were found to be significantly higher (at the 95% level) than those predicted in non-endemic areas and especially in aggregates of local endemic areas (Inaba *et al.*, 1977). Positive correlations were found between these prevalence rates and liver cancer rates in individual local areas in 1968–72, which were significant at the 95% level only for men (Table 3). The correlations for men increased in the period 1970–75, and while the correlation for women in that period became positive it remained compatible with chance. No adjustment was made for possible covariation with prevalence of hepatitis viral infection.

In a separate analysis analogous to that for liver cancer, Inaba (1982) assessed the frequency of mortality from other gastrointestinal malignancies in endemic areas by examining standardized mortality ratios in relation to those for Japan as a whole. No excess of cancer of the oesophagus, stomach, colon or rectum was noted for people of either sex, although the ratios of cancers of the bile duct and the pancreas in men were slightly but significantly elevated in endemic areas.

(*b*) *Cancers of the oesophagus and stomach*

In the study of Liu *et al.* (1983), significantly positive correlations were found for both stomach and oesophageal cancer for men and women in one province (Jiangxi), while the results for other provinces were inconsistent. No correlation was suggested in the analysis of 24 provinces with respect to stomach cancer. In another analysis (Guo *et al.*, 1984), no

Table 3. Descriptive studies of infection with *Schistosoma japonicum* and cancer

Reference	Population observed	Outcome index	Exposure index	Geographical correlations
Inaba *et al.* (1977)	Japan, Yamanashi Prefecture, localities	HCC mortality rate, 1968–72, 1970–75	Prevalence of schistosomiasis, both sexes, 1958–62	1968–72, males: 0.303*; females: -0.067 1970–75, males: 0.463*; females: 0.236
	Japan, Yamanashi Prefecture, endemic *versus* non-endemic areas	HCC mortality rate, 1970–75	Prevalence of schistosomiasis, both sexes, 1958–62	SMR, endemic males, 156 ± 21 females, 148 ± 26
				SMR, non-endemic males, 127 ± 17 females, 128 ± 21
Liu *et al.* (1983)	China, 24 provinces	Stomach cancer mortality rate	Schistosomiasis mortality rate	Not correlated
		Liver cancer mortality rate	Schistosomiasis mortality rate	Not correlated
		Colorectal cancer mortality rate	Schistosomiasis mortality rate	Males, $r = 0.695, p < 0.001$; females, $r = 0.625, p < 0.005$
	China, 10–98 counties of six high endemicity provinces	Stomach cancer mortality rate	Schistosomiasis mortality rate	Males, significant positive correlation in three provinces Females, positive correlation in four provinces ($p < 0.05$ in two)
		Oesophageal cancer mortality rate	Schistosomiasis mortality rate	Males, significant positive correlation in two provinces Females, positive correlation in five provinces ($p < 0.05$ in one)
		Liver cancer mortality rate	Schistosomiasis mortality rate	Males, significant positive correlation in one province; Females, significant positive correlation in five provinces ($r = 0.22$, 0.24, 0.32, 0.39, 0.44)
		Colorectal cancer mortality rate	Schistosomiasis mortality rate	Males, $r = 0.36$, 0.49, 0.58, 0.71, 0.81, 0.89 (all $p < 0.05$) Females, $r = 0.23$, 0.41, 0.44, 0.74, 0.85, 0.85 (all $p < 0.05$)

Table 3 (contd)

Reference	Population observed	Outcome index	Exposure index	Geographical correlations
Guo *et al.* (1984)	China, 7 counties of Jiangsu Province	Stomach cancer mortality rate	Schistosomiasis mortality rate	$r = -0.268, p < 0.001$ Inverse correlation with infection prevalence rate
		Oesophagus mortality rate	Schistosomiasis mortality rate	$r = 0.059, p \geq 0.20$
		HCC mortality rate	Schistosomiasis mortality rate	$r = 0.0053, p \geq 0.50$
		Colorectal cancer mortality rate	Schistosomiasis mortality rate	$r = 0.630, p < 0.001$ Direct correlation with infection prevalence rate
Xu & Su (1984)	China, 89 communes in 4 high-prevalence counties, Jiangsu Province 1977–79	Colorectal cancer mortality rate	Estimated *S. japonicum* infection prevalence rate	$r = 0.68, p < 0.01$
	24 communes, Haining county, Zhejiang Province	Colorectal cancer incidence rate	*S. japonicum* survey prevalence rate	$r = 0.60, p < 0.01$ (separately, colon, $r = 0.42$; rectum, $r = 0.48$)
Guo *et al.* (1985)[a]	China, Haining county, Zhejiang Province	Colorectal cancer mortality rate	*S. japonicum* survey prevalence rates	–
Li (1988)	China, 12 provinces in south	Colorectal cancer mortality	Incidence rate of schistosomiasis	$r = 0.71, p < 0.01$
	10 counties of Jiaxing area of Zhejiang Province	Colorectal cancer mortality	Incidence rate of schistosomiasis	$r = 0.90, p < 0.001$
	4 groups of counties in Jiaxing Prefecture	Colorectal cancer mortality	Incidence rate of schistosomiasis	$r = 1.00, p > 0.05$
Guo *et al.* (1993)	China, 49 rural counties selected on the basis of diversity of mortality from selected cancers	Colorectal cancer mortality rate	Schistosomiasis mortality rate	Univariate: males, $r = 0.395$, $p < 0.01$; females, $r = 0.538$, $p < 0.01$ Multivariate standardized: males, $r = 0.333, p < 0.01$; females, $r = 0.537, p < 0.01$

HCC, hepatocellular carcinoma; *, significant

[a]Correlation not formally tested

positive correlation between the prevalence of infection and mortality from either stomach or oesophageal cancer was found in the counties in Jiangsu Province.

(*c*) *Colorectal cancer*

In the study of Liu *et al.* (1983), mortality from colorectal cancer was correlated with that from schistosomiasis (r = 0.695 for men and 0.625 for women) in 24 Chinese provinces. In the analysis by county, significantly positive correlations were found for people of each sex in all six provinces (r, 0.23–0.89; median, 0.61).

Colorectal cancer mortality was correlated with 'prevalence of infection' (r = 0.63 for the two sexes combined) in seven counties in Jiangsu (Guo *et al.*, 1984); and the prevalence of infection was correlated with cancer mortality (r = 0.68) in the 89 communes of four high-prevalence counties in the Province (Xu & Su, 1984) and with cancer incidence (r = 0.42 for colon, 0.48 for rectum, 0.60 overall) in 24 communes of Haining County, Zhejiang Province (Xu & Su, 1984). Mortality from colorectal cancer was correlated with the incidence of schistosomiasis in 12 provinces of South China (r = 0.71), in 10 counties of the Jiaxing area of Zhejiang Province (r = 0.90) and in four county groups in Zhejiang Province (r = 1.00) (Li, 1988). Although in the latter analyses concern was raised about covariation between schistosomal infection and low levels of dietary selenium, in none of the above were dietary or other possible causes of colorectal cancer taken into consideration.

In a large correlation study from China, 65 rural counties were selected on the basis of the diversity of mortality rates from selected malignancies in an attempt to examine links between cancer mortality in 1973–75 and the dietary habits in 1983 of carefully selected, representative inhabitants (Chen *et al.*, 1990). The correlation between mortality rates for colorectal cancer and those for schistosomiasis was formally examined in a regression analysis, with adjustment for estimated consumption of individual nutrients and micronutrients. A significant association (r = 0.89, $p < 0.001$) was found. The correlation was significant for mortality from cancers of both colon (0.72) and rectum (0.88) when they were analysed in a subset of 49 counties. In both studies, the strength of the relationship between mortality from schistosomiasis and from cancer was as strong and consistent as that between mortality from schistosomiasis and any other variable. In a separate analysis of mortality from colon cancer by sex, significant associations with mortality from schistosomiasis were found for both men and women (Guo *et al.*, 1993).

While decades have passed since the first substantial efforts were made to control *S. japonicum* infection, no serious attempt has been made to assess the impact of eradication on the incidence of colorectal cancer. In one area, the continued high incidence of colorectal cancer has been attributed to the large number of people with controlled, advanced schistosomiasis (Guo *et al.*, 1985).

2.2 Case reports and case series

The first suggestion of a link between schistosomiasis and cancer came from careful assessment of clinical and pathological observations (Goebel, 1905; Ferguson, 1911; Kazama, 1921); however, as knowledge of the distribution and presentation of both schistosomiasis and cancer has accumulated, it has become apparent that case reports and

series cannot help in assessing cancer etiology. In endemic areas, substantial proportions of the population are infected. Moreover, evidence of infection is widely disseminated throughout the body, remains there throughout life and may or may not produce symptomatic disease. Under the null hypothesis of no association between infection and cancer occurrence, it is therefore to be expected that a substantial proportion of the population of all ages will have been among those with clinical or subclinical disease, that a substantial proportion of patients with newly diagnosed cancer will show evidence of past infection, that evidence of infection may appear in virtually any organ of the body, and that such evidence of infection may therefore be expected to be incorporated in or found adjacent to virtually any tumour. Nonetheless, cases and case series can add credibility to the evidence of a causal relationship between these infections and cancer by documenting the anatomical proximity of the effects of infection to the appearance of the malignancy and by illustrating changes in the clinical and pathological characteristics of malignancies as they appear in conjunction with the infection.

2.2.1 *Schistosoma haematobium*

Subsequent to the early reports, large series of cases of urinary bladder cancer have been reported in association with evidence of *S. haematobium* infection (see Box).

The case descriptions have repeatedly emphasized the preponderance of squamous-cell urinary bladder tumours among cases with evidence of schistosomal infection, the somewhat different distribution over the surface of the bladder (notably the rarity of occurrence in the trigone) in comparison with bladder tumours in developed countries, and the prevalence of metaplastic changes in conjunction with evidence of infection (da Silva Lopes, 1984). Clinically, the most notable and consistent feature described in these series is the relative youth of the cases with evidence of a link to *S. haematobium* infection. While this observation is made in almost all of the reports, and is usually interpreted as constituting evidence of etiological heterogeneity, the finding does not constitute strong evidence because evidence of the infection is known to decrease in frequency with age.

Other than urinary bladder cancer, the malignancies most frequently reported in association with *S. haematobium* infection are those of the female genitalia. A few dozen cases of squamous cervical carcinoma have been reported from endemic areas (Badawy, 1962; Youssef *et al.*, 1962; Berry, 1966; Sharma *et al.*, 1970; Youssef *et al.*, 1970; Bognel *et al.*, 1980; Schwartz, 1984; El Tabbakh & Hamza, 1989), and the same authors and others (Shafeek, 1957; Iskander & Kamel, 1968; Sunder-Raj, 1976; Al-Adnani & Saleh, 1982; El-Maraghy *et al.*, 1982) have reported certain other genital squamous malignancies, ovarian cystadenocarcinomas, Brenner tumours and teratomas. It has been alleged that breast cancers in men infected with *S. haematobium* constitute a relatively high proportion of all male breast cancers in Egypt (El-Gazayerli & Abdel-Aziz, 1963; Sherif *et al.*, 1980), but the reported numbers are small and cannot be evaluated. Relatively small numbers of other malignancies that have been reported in association with evidence of *S. haematobium* infection include hepatocellular carcinoma (Nkrumah, 1964; Hashem, 1971), bladder sarcoma (Alwan *et al.*, 1988) and lymphomas (Edington *et al.*, 1970; Cheever *et al.*, 1978).

Angola (da Silva Lopes, 1984)
Egypt (Mohamed, 1954; Mustacchi & Shimkin, 1958; El-Gazayerli & Khalil, 1959; Hashem *et al.*, 1961; Aboul Nasr *et al.*, 1962; Makhyoun *et al.*, 1971; El-Bolkainy *et al.*, 1972; Khafagy *et al.*, 1972; El-Sebai, 1980; El-Bolkainy *et al.*, 1981; Christie *et al.*, 1986a; Tawfik, 1988; Fukushima *et al.*, 1989)
Senegal (Quenum, 1967)
Zambia (Bhagwandeen, 1976; Elem & Purohit, 1983)
Nigeria (Attah & Nkposong, 1976), Malawi (Lucas, 1982b)
Sudan (Malik *et al.*, 1975; Sharfi *et al.*, 1992)
Kenya (Anjarwalla, 1971; Bowry, 1975)
Iraq (Al Adnani & Saleh, 1983; Al-Fouadi & Parkin, 1984)
Natal (Cooppan *et al.*, 1984)
South Africa (Transvaal) (Higginson & Oettlé, 1962; Hinder & Schmaman, 1969; Kisner, 1973)
Uganda (Dodge, 1962)
Saudi Arabia (Cutajar, 1983; Hanash, 1984; Khurana *et al.*, 1992)
Kuwait (Al-Shukri *et al.*, 1987)
Mozambique (Prates & Gillman, 1959; Gillman & Prates, 1962; Ebert, 1987)
United Republic of Tanzania (Kitinya *et al.*, 1986)
Zimbabwe (Houston, 1964; Gelfand *et al.*, 1967; Thomas *et al.*, 1990) and
Among immigrants in Europe (Wagenknecht, 1974; Pieron *et al.*, 1983; Delmas *et al.*, 1986) or visitors to Africa (Diaz Hernandez *et al.*, 1984).

2.2.2 *Schistosoma mansoni*

Cases of liver cancer have been reported in connection with evidence of *S. mansoni* infection from Egypt (Hashem, 1971), Mozambique (Prates & Torres, 1965), Brazil (Cheever & Andrade, 1967; Lyra *et al.*, 1976), Puerto Rico (Martinez-Maldonado *et al.*, 1965), Saudi Arabia (Nouh *et al.*, 1990) and Nigeria (Edington *et al.*, 1970). Similarly, cases of colorectal cancer have frequently been described from Egypt (Afifi, 1948; Dimmette *et al.*, 1956; Cheever *et al.*, 1978) and Lebanon (Uthman *et al.*, 1991). Andrade and Abreu (1971) reported the occurrence of eight giant follicular lymphomas in 863 spleens removed from patients with portal hypertension due to infection with *S. mansoni*; subsequently, six additional cases of this neoplasm were described (Paes & Marigo, 1981) in a similar series of 714 spleens. Of these 14 lymphomas, four were further confirmed in biopsy samples or at autopsy; the rest were lost to follow-up. Although other individual cases of diverse lymphomas have been reported in patients with schistosomiasis (Andrade & Abreu, 1971; Cheever *et al.*, 1978; de Andrade *et al.*, 1982; Chirimwami *et al.*, 1991), no reports of giant follicular-cell lymphoma have subsequently appeared.

Other malignancies that have been reported in association with evidence of *S. mansoni* infection include prostatic cancer (Alexis & Domingo, 1986; Godec *et al.*, 1992), ovarian

teratoma (Kahn *et al.*, 1978), uterine leiomyosarcoma (Joyce *et al.*, 1972), renal-cell carcinoma (Oro Ortiz *et al.*, 1991), rectal carcinoid tumour (Satti *et al.*, 1988) and cancer of the cervix (Coelho *et al.*, 1979; Wright *et al.*, 1982).

2.2.3 *Schistosoma japonicum*

Most of the cases or series of cases of liver cancers reported in association with *S. japonicum* infection have come from Japan (Iuchi *et al.*, 1971; Nakashima *et al.*, 1975; Kojiro *et al.*, 1986; Fujimoto *et al.*, 1989; Kitani & Iuchi, 1990; Uetsuji *et al.*, 1990). Within such series, cases of liver cancer have been reported to occur commonly in patients who responded positively to a skin test or were shown histologically to have *S. japonicum* infection (Iuchi *et al.*, 1971; Nakashima *et al.*, 1975; Kojiro *et al.*, 1986); in patients who had evidence of hepatitis viral infection (Nakashima *et al.*, 1975; Kitani & Iuchi, 1990; Kojiro *et al.*, 1986); and in those with schistosoma-associated cirrhosis (Iuchi *et al.*, 1971; Kitani & Iuchi, 1990). In one small series from an endemic area, *S. japonicum* was not found to be especially common in cases of liver cancer (Kamo & Ebato, 1982).

Series of cases of gastric cancer associated with histological evidence of *S. japonicum* infection have been reported from both Japan (Amano, 1980) and China (Wang, 1979; Qian & Yi, 1980; Feng & Shi, 1981; Wang & Kuang, 1983; Zhou, 1986).

Series of cases of colorectal cancer found in association with infection with *S. japonicum* have been reported from Japan (Shindo, 1976; Inoguchi *et al.*, 1978; Naito *et al.*, 1979; Amano, 1980; Hashimoto *et al.*, 1986; Sekiguchi *et al.*, 1989), the Phillipines (Abanilla, 1986) and China (Chen & Chen, 1957; Tsou & Ying, 1958; Wu *et al.*, 1960; Chuang *et al.*, 1979; Chen *et al.*, 1980; Chen *et al.*, 1981; Zhao & Wong, 1981; Liu *et al.*, 1983; Zhuang *et al.*, 1985; Chen, 1986). As in studies of bladder cancer, schistosomally infected patients are of younger average age in most series (Abanilla, 1986; Chen, 1986). This observation is difficult to interpret in the light of differences in the prevalence of infection with age.

Other malignancies that have been reported as individual cases in relation to *S. japonicum* infection include squamous-cell carcinoma of the skin (Ohtake *et al.*, 1991), malignant schwannoma (Schwartz, 1982), carcinoma of the parotid gland (Tangchai & Poshayalakshana, 1968), bronchogenic carcinoma (Ishihara *et al.*, 1984) and breast cancer (Zhou, 1983).

2.3 Cohort study

Inaba (1984) categorized all 2067 people native to a locality in Yamanashi Prefecture, Japan, endemic for *S. japonicum* infection into four classes, depending on whether they had resided before 1957 in that place for more than 50 years, 30–49 years, 10–29 years or fewer than 10 years (Table 4). Duration of residence was taken as an indicator of extent of exposure. They were then followed in the locality-based registers available in Japan, and all death certificates were collected. There were 26 deaths from liver cancer and 16 from colorectal cancer (nine from colon cancer). It was found that men who had lived for more than nine but less than 50 years in a community had a significantly high risk of liver cancer, and that women living in the community for 50 or more years had a significantly high risk of colorectal cancer. No adjustment was made for diet or for hepatitis viral infection.

Table 4. Cohort study of cancer based on death certificates for natives of a town in Yamanashi Prefecture, Japan, endemic for *S. japonicum* infection

Length of residence before 1957 (years)	Number of exposed subjects	Cancer	Number of cases	SMR	
				Males	Females
0–9	428	Liver	1	0.81	–
10–29	575		9	3.2[a]	2.5
30–49	655		10	2.9[a]	1.1
≥ 50	404		6	1.8	0.88
0–9	428	Colon	0	–	–
10–29	575		2	–	2.0
30–49	655		4	2.4	1.9
≥ 50	404		3	–	4.6[a]

From Inaba (1984); SMR, standardized mortality rate
[a] 95% confidence interval excludes 1.0

2.4 Case–control studies (with retrospective exposure assessment)

2.4.1 *Schistosoma haematobium*

Mustacchi and Shimkin (1958) identified 48 male and 7 female hospitalized patients with urinary bladder cancer in the Egyptian Nile Delta city of Tanta among 1472 consecutive admissions to the hospital. All patients were evaluated in relation to the presence of *S. haematobium* eggs in a urine sample taken at admission and to any subsequent evidence of *S. haematobium* infection [the latter but not the former could have been obtained on the basis of knowledge of the presence of bladder cancer]. After multivariate adjustment for age, sex and urban or rural origin, odds ratios of 2.1 ($p = 0.04$) were seen for the finding of eggs at the time of admission and 2.2 ($p < 0.01$) for any subsequent evidence of schistosomal infection.

Prates and Gillman (1959) compared 100 urinary bladder cancer cases in Maputo, Mozambique, with 185 cases found at autopsy in people over 40 years of age with respect to the frequency of identification of *S. haematobium* eggs in relation to the histological type of bladder cancer. Eggs were found in 33 of the cases found at autopsy and in 61% of controls [odds ratio, 0.3; 95% confidence interval (CI), 0.2–0.5]. Eggs were found in 56% of the 59 squamous-cell cancer patients but in none of the transitional-cell cancer patients. [The methods used to examine the biopsy and autopsy specimens were dissimilar, and there was no reconciliation of the high rate in cadavers, despite the absence of eggs in the bladders of people with transitional-cell cancer. The causes of death of the controls were not described, and no adjustment was made for differences in specific age or place of origin.]

Hinder and Schmaman (1969) compared the prevalence of histologically identified eggs in punch biopsy specimens from 79 patients with urinary bladder carcinoma in Johannesburg, South Africa, with the prevalence in two or more full-thickness biopsy specimens from 101 people over the age 15 who came to autopsy. Eggs were identified in 34.2% of the cases but in only 9.0% of the autopsied patients [odds ratio, 5.3; 95% CI,

2.3–12]. The causes of death of the controls were not provided, and no adjustment was made for differences in specific age or place of origin. When cases were analysed by histological type, 19% of transitional-cell carcinomas and 68% of squamous-cell carcinomas contained eggs.

Gelfand *et al.* (1967), in Harare, Zimbabwe, compared 33 patients with urinary bladder cancer with other hospital patients who had been 'submitted to similar investigation' and were matched on age, sex and race. Comparisons were made on the basis of the results of pelvic X-rays (33 pairs) and rectal biopsies (31 pairs). Among the 16 pairs discordant for calcified eggs identified by X-ray, the case was positive in 15, giving an odds ratio of [15; 95% CI, 2.0–114]; among the 15 pairs discordant for the results of rectal biopsy, the case was positive in 13, giving an odds ratio of [6.5; 95% CI, 1.5–29]. The diagnoses of disease in the controls were not described, and no adjustment was made for differences in smoking habits or place of origin.

In a project for cytological screening of urinary bladder cancer conducted from 1976 to 1979 in a location in the Nile Delta highly endemic for *S. haematobium*, participants over 20 years of age were characterized by occupation, on the presumption that the 4769 agricultural labourers in this age group had a higher prevalence of infection than 1112 people with other occupations (El-Bokainy *et al.*, 1982). All 10 cases of bladder cancer detected and confirmed histologically appeared among the agricultural workers [prevalence ratio, ∞]. Although the ages of the subjects were not analysed in detail, it was concluded that adults of this working class were at increased risk of bladder cancer.

In Zambia, Elem and Purohit (1983) compared the bladders of 50 patients who had died of urinary bladder cancer with bladders from age- and sex-matched cadavers (mostly trauma victims matched on age and sex to the decedent) by means of X-ray examination and digestion of tissues away from the eggs they contained. The bladders of the cases were [3.8] [95% CI, 1.4–10] times as likely to show schistosomiasis by X-ray and [14] [95% CI, 4.6–43] times as likely to contain *S. haematobium* eggs.

In the Bulawayo region of Zimbabwe, cancer registration procedures from 1963 to 1977 included collection of information about exposures, including past history of clinical schistosomiasis ('bilharzia' or 'blood in the urine') (Skinner *et al.*, 1993). Some difference in the availability of information about past schistosomiasis is evident between cases of urinary bladder cancer (61%) and cases of cancer of other types (50%). The exposures of 305 patients with bladder cancer were compared with those of 3145 other cancer patients, with and without exclusion of people with cancers known to be linked to smoking. The occurrence of bladder cancer was associated with place of origin, a lower level of education and a more menial occupation. No effect of smoking was found for squamous-cell cancers and only a modest effect (1.3) for other cancers. For a history of schistosomiasis in men, the odds ratio (using all other cancer cases as controls, relative to no such history and adjusted for age, tobacco use, province of origin, education and occupation) was 3.9 (95% CI, 2.9–5.1) for all types of cancer and 3.4 for both squamous-cell cancer and other specified carcinomas. When the cases of smoking-related cancers were excluded from the control group, the odds ratio for squamous-cell cancers increased to 3.9 and that for other cancers dropped to 3.1.

These studies are summarized in Table 5.

Table 5. Case–control studies of infection with *Schistosoma haematobium* and urinary bladder cancer

Reference	Location	Source of cases	Source of controls	Measure of exposure	No. of cases/ no. of controls	Cases/controls exposed (%)	Odds ratio	95% CI (or *p*)	Cases with squamous-cell tumours (%)
Mustacchi & Shimkin (1958)	Tanta, Nile Delta, Egypt		Other admissions to hospital	Eggs in first urine sample	55/1417	14.5/7.6	2.1[a]	0.04	Not specified
				All clinical evidence, including history and cystoscopy	55/1417	49.0/23.3	2.2[a]	< 0.01	
Prates & Gillman (1959)	Maputo, Mozambique		Autopsied people > 40 years	Eggs identified in histological sections	100/185	33/61.1	[0.3	0.2–0.5]	59 (56 with past exposure)
Hinder & Schmaman (1969)	Johannesburg, South Africa		Autopsied people > 15 years	Post-mortem punch biopsy sample	79/101	34.2/9.0	[5.3	2.3–12]	28 (68 with past exposure)
Gelfand *et al.* (1967)	Harare, Zimbabwe		Matched patients[b] of same age, sex, race, on different hospital ward	Pelvic X-ray	33/33	45.5/3.03	[15	2.0–114]	62 (62 with past exposure)
				Rectal biopsy	31/31	54.8/19.4 (discordant matched pairs 1) 15/1 2) 13/2)	[6.5	1.5–29]	
El-Bolkainy *et al.* (1982)	Dakahliya Governorate, Nile Delta, Egypt	Rural residents participating in a bladder screening programme subdivided by occupation		Occupation as farmer	10/5871	100/81	∞		50
Elem & Purohit (1983)	Lusaka, Zambia		Cadavers without malignancy (mostly traumatic death)	Digestion and centrifugation of bladder	50/50	94.0/40.0	[14	4.6–43]	72
				Pelvic X-ray	50/50	38/14	[3.8	1.4–10]	
Skinner *et al.* (1993)	Bulawayo, Zimbabwe	Cancer registry cases, males	Registry cases with other cancers	Self-reported history of bilharzia or blood in urine	305/3145	348/11.7	3.9[c]	[2.9–5.1]	71. No change when tobacco-related cancers excluded from controls

[a]Adjusted for age, sex and urban or rural residence

[b]Who were 'submitted to same procedure'

[c]Adjusted for age, period, province, drinking and smoking

Of interest is an additional case–control study of urinary bladder cancer, which was not performed to test the hypothesis of schistosomal etiology (Table 6). Makhyoun (1974) compared males admitted to hospital for urinary bladder cancer in Alexandria and Tanta, Egypt, with other admitted males, matched on age and smoking history, after stratification of cases and controls on the basis of history of clinical schistosomiasis. In 80% of people without such a history who had smoked heavily or moderately, the malignancies were strongly associated with cumulative smoking history. Only 23% of the schistosomiasis patients had smoked moderately or heavily, and the link between cancer and smoking in these subjects, while present, was weaker. [While the role of past clinical schistosomiasis in bladder cancer was not assessed within groups comparable for past smoking history, the low level of smoking among the patients with *S. haematobium*-associated cancer makes it extremely unlikely that the pattern of smoking could explain the strong links between infection and bladder cancer.]

Table 6. Case–control study of urinary bladder cancer in relation to smoking and history of infection with *Schistosoma haematobium* among males admitted to hospital

Infection status	Smoking index[a]	No. exposed		Odds ratio
		Cases	Controls	
History of *S. haematobium* infection	None	66	80	1.0
	Light (< 300)	149	145	1.3
	Moderate (300–600)	42	35	1.5
	Heavy (> 600)	21	18	1.4
	Moderate–heavy	63	53	1.4
No history of *S. haematobium* infection	None	15	23	1.0
	Light (< 300)	3	24	0.2
	Moderate (300–600)	41	27	2.3
	Heavy (> 600)	28	13	3.3[b]
	Moderate–heavy	69	40	2.6[b]

From Makhyoun (1974); odds ratios calculated by the Working Group.
[a]Daily number of cigarettes times number of years smoking
[b]Significantly different at $p < 0.01$

2.4.2 *Schistosoma japonicum*

In a comparison based on skin testing for antigens to *S. japonicum*, Iuchi *et al.* (1971) found that 85.2% of 52 cases of hepatocellular carcinoma and 68.2% of 217 other hospital in-patients over 40 years of age had antigens. No adjustment was made for evidence of past hepatitis viral infection.

Inaba *et al.* (1984) used skin testing and medical histories to compare 62 cases of liver cancer diagnosed in seven hospitals in an endemic area, Yamanashi Prefecture, Japan, in 1977–79 with age- and sex-matched hospital controls admitted for various diseases other than liver disease. While the univariate relative risk was 9.5 [95% CI, 2.2–41], restriction of the analysis to the 88 subjects seronegative for hepatitis B surface antigen and their controls gave a relative risk of [6.7; 1.5–30] for 39 alcohol users and of [4.7; 1.2–19] for 49 non-users.

Guo and Lu (1987) compared 166 patients who had died of liver cancer with 166 people who had died from other cancers and with 166 healthy people, both groups matched on age, sex and place of residence with respect to a history of *S. japonicum* infection. The matched odds ratio for schistosomal infection based on both series of controls was 2.2 ($p < 0.01$). Relative risks of [2.5; 95% CI, 1.4–4.4] and [2.3; 1.3–4.1] were found in relation to cancer decedents and healthy controls, respectively, after adjustment for smoking and family history of liver cancer but not for evidence of hepatitis viral infection. The relative risk estimates increased significantly with the interval since diagnosis of schistosomiasis, whether cancer or healthy controls were used. Dietary exposure to aflatoxins was considered not to be prevalent in this area.

Amano (1980) compared 362 patients with stomach cancer who were treated surgically in Yamanashi Prefecture, Japan, with 897 surgical cases with non-malignant disease of the stomach and duodenum, and found *S. japonicum* eggs [1.8] [95% CI, 1.3–2.6] times more frequently in the tissues of cases than in those of controls. No adjustment was made for potential confounders. In the same study, eggs were found [1.2] [0.62–2.5] times more often in the tissues of 103 colon cancer cases than in the 96 controls with benign disease of the colon. No adjustment was made for diet or other potential confounders.

In endemic Kunshan County in Jiangsu Province, China, Xu and Su (1984) gathered medical histories on schistosomiasis for all colorectal cancer patients and for patients with other cancers and from healthy neighbours, each matched on age, sex, occupation and work unit. While no significant association was found between colon cancer and past history, odds ratios of 8.3 and 4.5 were found for rectal cancer in comparisons with cancer controls and healthy controls, respectively. No adjustment was made for diet or other potential determinants of colorectal cancer.

In the same county, Guo *et al.* (1987) compared people who had died of colon cancer with those who had died of lung cancer and with healthy people, with respect to any medical history of early- or late-stage schistosomiasis. In relation to healthy controls, odds ratios of 2.4 [CI not given] were found for a history of early-stage infection and 5.5 [CI not given] for a history of late-stage schistosomal disease. After adjustment for smoking and a family history of colon cancer, but not for diet or exercise, significant associations were still found: 2.1 (95% CI, 1.1–3.8) and 4.2 (1.2–15) in relation to lung cancer controls for early- and late-stage disease, respectively, and 2.4 (1.1–5.0) and 5.7 (1.3–25) for the same exposures in relation to healthy controls. The risk was found to increase stepwise from 1.2 to 4.3 after < 10 years to > 30 cumulative years of infection [CI not given].

These studies are summarized in Table 7.

A number of studies have addressed the association between infections with *S. mansoni* and *S. japonicum* and cancer of the liver. The possible confounding of schistosomal infection with hepatitis viral infection (see IARC, 1994) in these studies has rarely been addressed empirically. A recent review of the coincidence of infection with hepatitis B virus and with *S. mansoni* and *S. japonicum* in population-based studies (Chen *et al.*, 1993) showed no significant increase in the prevalence of hepatitis B surface antigenaemia in people with these schistosomal infections. The prevalence of joint infection is, however, higher in hospital patients than in members of the corresponding general population; in particular, patients hospitalized with hepatosplenic schistosomiasis are more likely to be seropositive

Table 7. Case–control studies of infection with *Schistosoma japonicum* and cancer

Reference	Location	Source of cases	Source of controls	Measure of exposure	Number of cases/controls	Exposure in cases/controls	Odds ratio	95% CI
Liver cancer								
Iuchi *et al.* (1971)	Kofu, Yamanashi Prefecture, Japan	Previous diagnoses in hospital autopsies	Other in-patients	Skin test for *S. japonicum*	61/303	Prevalence of + 91.8%/53.1%	[9.9]	[3.9–25]
				Histology	61/21	91.8%/71.4%	[4.5]	[1.2–17]
Inaba *et al.* (1984)	Yamanashi Prefecture, Japan	Diagnoses in 7 hospitals, 1977–79	Patients matched on sex, age, hospital	Skin test for *S. japonicum*; medical history	62/62		9.5	[2.2–41]
						Negative for hepatitis B surface antigen, alcohol use	[6.7]	[1.5–30]
						Negative for hepatitis B surface antigen, no alcohol use	[4.7]	[1.2–19]
Guo & Lu (1987)	Kunshan County, Jiangsu Province, China	Liver cancer deaths, 1982–83	Deaths from other cancers 'Healthy people', matched on age, sex, county of residence	History of infection	166/166/166		Matched odds ratio, 2.2 ($p < 0.01$); unmatched [1.9 for cancer controls],	[1.1–3.3]
							[2.1 for healthy controls]	[1.2–3.7]
							After adjustment for smoking and family history of liver cancer, odds ratio of [2.5] for cancer controls	[1.4–4.4]
							and [2.3] for healthy controls	[1.3–4.1]
Stomach cancer								
Amano (1980)	Kofu, Yamanashi Prefecture, Japan	Surgically treated hospital cancer patients	Non-malignant cases	*S. japonicum* eggs in pathological specimens	362/897	15.2%/9.0% prevalence	[1.8]	[1.3–2.6]

Table 7 (contd)

Reference	Location	Source of cases	Source of controls	Measure of exposure	Number of cases/controls	Exposure in cases/controls	Odds ratio	95% CI
Colorectal cancer								
Amano (1980)	Kofu, Yamanashi Prefecture, Japan	Surgically treated hospital colon cancer patients	Non-malignant cases	*S. japonicum* eggs in pathological specimens	103/96	22.3%/18.8% prevalence. Much higher differential among those aged 40–49	[1.2]	[0.62–2.5]
Xu & Su (1984)	Kunshan County, Jiangsu Province, China	Colorectal cancer cases, 1973–79	Non-gastrointestinal cancer patients Neighbours, each matched on age, sex, occupation and production brigade or team	Medical history from patients, relatives, barefoot doctors	98/98/98 (colon) 154/154/154 (rectum)		Colon: odds ratio, 1.2 with other cancer controls; 0.64 with healthy neighbourhood controls Rectum: odds ratio, 8.3 with other cancer controls and 4.5 with healthy neighbourhood controls	[0.48–3.2] (triplets) [0.33–1.2] [3.1–22.6] [1.7–12.1]
Guo *et al.* (1987)	Kunshan County, Jiangsu Province, China	Colon cancer deaths, 1981–83	Lung cancer patients 'Healthy persons'	Medical history	197/205/200		Odds ratio, 2.4 (early-stage disease), 5.5 (late-stage disease) After adjustment for smoking and family history of colon cancer, but not diet or exercise, overall significant association remains: 2.1 and 4.2 for early- and late-stage disease with lung cancer controls, 2.4 and 5.7 with healthy controls After < 10, -20, -30, ≥ 30 years since diagnosis, 1.2, 1.9, 2.9, 4.3 duration–response effects	

for hepatitis B surface antigen than those with latent or intestinal schistosomiasis. These observations suggest that hepatitis B viral infection may confound the association between schistosomal infection and liver cancer in hospital-based studies of individuals. There are no similar data that would allow evaluation of the possibility of confounding between hepatitis C viral infection and schistosomal infection.

3. Studies of Cancer in Animals

3.1 Infection with *Schistosoma haematobium* alone

3.1.1 *Mouse*

Groups of 6–10 male C3H mice, two months old, received single subcutaneous injections of 1.5–10 mg of lyophilized *S. haematobium* eggs or worms in saline or tricaprylin. Another group received a single intraperitoneal injection of 4 mg of egg material, while a further group received two subcutaneous injections of 3 or 4 mg of egg material at an interval of three months. Animals were examined for the presence of tumours 13, 17 and 20 months after injection. The experiment was terminated at 20 months. No tumours were seen at the injection site. Of the 20 mice killed at termination, five had pulmonary tumours and three had hepatomas. The authors noted that the frequency of pulmonary and liver tumours was similar to that in historical controls (Shimkin *et al.*, 1955). [The Working Group noted the limited reporting and that an infectious agent was not employed.]

As part of an experiment on *S. haematobium* in combination with 2-acetylaminofluorene (see below), a control group of 20 Swiss mice [sex and age unspecified] was repeatedly treated by subcutaneous injection with cercariae [schedule unspecified] and kept for 44 weeks. Urinary bladder epithelial hyperplasia beginning as early as three weeks was observed in the majority of the mice. Hyperplasia was not observed in 100 untreated control animals (Hashem & Boutros, 1961). [The Working Group noted the absence of ova or worms in the bladder and the short duration of the study.]

A group of 30 male BALB/c mice, three to four weeks of age, received subcutaneous implants of ligated urinary bladder cysts from donor mice. Bladder cysts were prepared by distending bladders with 0.15 ml mineral oil containing 1000 lyophilized *S. haematobium* ova before ligation. The mice were observed for 44 weeks. A control group of 29 mice received donor bladder cysts containing 0.15 ml mineral oil alone. Hyperplasia of the bladder cyst epithelium was seen in 4/30 mice and 5/29 controls. One transitional-cell tumour and one squamous-cell tumour in the implanted bladder cyst [tumour pathology not described] were reported in the experimental group but not in controls (Al-Hussaini & McDonald, 1967). [The Working Group noted the unusual design of the experiment and the lack of significance of the tumour response.]

3.1.2 *Rat*

In a combination experiment, a group of 100 white rats [sex, strain and age unspecified], weighing 140–160 g, were exposed to water containing 2000 *S. haematobium* cercariae per

litre. The urinary bladders from half of the rats were examined after 12 months, and the remaining rats were examined at 24 months. No malignant change was reported; a few rats had bladder lesions reported as 'sessile polyps' (Gawish, 1975). [The Working Group noted the inadequate reporting and found no compelling evidence for sustained bladder infection.]

3.1.3 *Hamster*

In a combination experiment reported in a proceedings volume (James *et al.*, 1974), a group of 50 hamsters [sex unspecified] were exposed to 80 *S. haematobium* cercariae. No adverse pathological finding in the urinary bladder was reported in the 56-week experiment. [The Working Group noted the inadequate reporting on e.g. the presence of infection.]

A group of 18 male hamsters, eight weeks of age, was exposed to *S. haematobium* by immersion in water containing 250 cercariae for 1 h. Exposure was repeated three months later. The animals were killed 7–11 months after the first exposure, and the viscera were examined histopathologically. Eleven animals developed manifestations of schistosomal cystitis. In four animals, epithelial hyperplasia of the urinary bladder was related to sites of submucosal reaction to ova. In four other animals, the bladder epithelial changes consisted of both hyperplasia and squamous metaplasia (El-Morsi *et al.*, 1975). [The Working Group noted that hyperplasia of the bladder is unusual in untreated hamsters and that the duration of observation was short in comparison with the lifespan of the animals, so that tumours might have developed in the animals if they had been allowed to live longer.]

3.1.4 *Opossum*

Eight opossums (*Didelphis marsupialis*) were exposed to 1000–2000 cercariae of *S. haematobium* on the shaved skin for 30 min. Between 18 and 53 weeks after infection, two animals were reported to have mucosal fibrous plaques in the urinary bladder. A third animal had multiple epithelial lesions of the bladder that were variably described as hyperplastic, papillomatous, polypoid or tumourous. The presence of eggs was associated with the lesions in two animals, and all three animals had evidence of infection (Kuntz *et al.*, 1971).

3.1.5 *Nonhuman primate*

Young adult primates, including one talapoin (*Cercopithecus talapoin*), seven capuchins (*Cebus apella*), seven squirrel monkeys (*Saimiri sciureus*) and 11 African baboons (*Papio cynocephalus*), were exposed percutaneously to 1000–2000 cercariae of *S. haematobium* and observed for up to 24 months. Epithelial lesions of the urinary bladder, reported to be papillary transitional-cell carcinomas, were found in a talapoin monkey which died 21 weeks after infection and in a capuchin that was killed 56 weeks after infection. An epithelial lesion reported as a papilloma of the ureter was associated with the presence of schistosomal eggs in an African baboon killed one year after infection (Kuntz *et al.*, 1972).

In a further experiment, nine capuchin monkeys (*Cebus apella*) were exposed via the skin to 1000–2000 cercariae of *S. haematobium* and were examined for pathological changes in the urinary bladder by laparotomy and cystotomy 94–164 weeks after infection. Six of nine animals showed papillary hyperplasia with or without nodular hyperplasia. In two animals, only focal nodular hyperplasia was seen (Kuntz *et al.*, 1978).

In a study to detect C-type viral particles in tumours, it was reported that four of six capuchin monkeys that had been infected experimentally with *S. haematobium* developed lesions described as papillary carcinomas of the urinary bladder during periods of observation of 109–111 weeks. Three of the animals also had squamous metaplasia of the bladder epithelium (Kalter *et al.*, 1974). [The Working Group noted the lack of experimental details and of documentation of the pathological findings.]

Kuntz *et al.* (1975) described the pathological findings and parasitological and radiological observations in two gibbons (*Hylobates lar*) infected by skin application with 1000 cercariae of *S. haematobium*. Both animals developed evidence of infection, the most striking change being extensive calcification of the eggs in the urinary bladder. One animal had evidence of papillary and nodular transitional-cell hyperplasia of the bladder, and the other had similar lesions in the ureter. The lesions were described as morphologically similar to the grade-I and grade-II papillary transitional-cell carcinomas that are seen in the bladders of humans. [The Working Group noted the small number of animals and the equivocal diagnoses of the lesions.]

In combination experiments with baboons (*Papio* sp.) (Hicks *et al.*, 1980; Hicks, 1982), five animals were infected by an abdominal pouch method with 1000 cercariae of *S. haematobium* and kept for 2.5 years. Four animals had polypoid hyperplasia of the urinary bladder and one had endophytic papillary hyperplasia of the ureter. None of these lesions was considered to be a tumour.

3.2 Infection with *Schistosoma haematobium* in combination with administration of known carcinogens

3.2.1 *2-Acetylaminofluorene*

Mouse: Two groups of 20 Swiss mice [sex and age unspecified] were administered 0.2 ml of a 1.5% suspension of 2-acetylaminofluorene (2-AAF) in olive oil by stomach tube three times a week [duration unspecified]. One of the groups was repeatedly infected with *S. haematobium* cercariae by subcutaneous injection [dosing schedules and duration unspecified]. Animals were observed up to 44 weeks, at which time survivors were killed. A third group of 20 mice was infected with *S. haematobium* alone. Epithelial hyperplasia of the urinary bladder was observed in *S. haematobium*-infected mice. One of the 2-AAF-treated mice developed a benign villous papilloma of the bladder after 43 weeks. Four of the carcinogen-treated animals infected previously with *S. haematobium* developed bladder neoplasms at 36–44 weeks; one had an anaplastic infiltrating carcinoma and three had papillomas, two of which had malignant areas (Hashem & Boutros, 1961). [The Working Group noted the short duration, the lack of verification of infection and inadequate documentation of experimental details.]

Rat: A group of 100 white rats [sex, strain and age unspecified], weighing 160 g, were exposed to water containing 2000 *S. haematobium* cercariae per litre; 45 days after exposure, the rats received intraperitoneal injections of 50 mg/kg bw 2-AAF three times per week for four weeks, followed by a diet containing 0.06% 2-AAF and 1.6% indole for one year. A control group of 100 rats received the carcinogen alone. Ten rats were killed every two months and the bladders examined microscopically. In 80 rats in the combined group killed

after six months, all but five had transitional-cell carcinomas of the urinary bladder, as did 7 of the 10 control animals treated with 2-AAF and killed after 10 months (Gawish, 1975). [The Working Group noted the inadequate experimental design, the lack of verification of infection and the fact that the results for the two groups did not differ statistically.]

3.2.2 ortho-*Aminoazotoluene*

Hamster: In a study reported in a proceedings volume (James *et al.*, 1974), groups of 50 hamsters were exposed to 80 *S. haematobium* cercariae. Ten weeks later, 0.02 or 0.1% *ortho*-aminoazotoluene was incorporated into the diet. Administration of the carcinogen alone caused hyperplasia of the urinary bladder epithelium. In the combined group at the 0.1% dose level, malignant changes were seen in the bladder within 24 weeks . [The Working Group noted the inadequate reporting.]

3.2.3 N-*Nitrosamines*

Hamster: In a combination study reported as an abstract (Hicks *et al.*, 1977), groups of hamsters received a single intravesicular instillation of *N*-methylnitrosourea and were infected with *S. haematobium*. Urinary bladder tumours developed in 5/16 hamsters receiving the combined treatment, 0/26 uninfected controls [$p < 0.001$; Fisher exact test], 0/28 infected animals and 0/19 hamsters treated with *N*-methylnitrosourea alone. In groups of hamsters treated with *N*-nitrosobutyl-4-hydroxybutylamine (NBHBA), bladder tumours developed in 9/24 infected hamsters and 5/30 uninfected controls [$p = 0.057$; Fisher exact test]. [The Working Group noted the inadequate reporting.]

Nonhuman primate: In an experiment designed to simulate the possible proliferative stimulus of *S. haematobium* infection on cancer growth due to exposure to low doses of *N*-nitrosamines in humans, small groups of baboons (*Papio* sp.) were either infected through an abdominal pouch with 1000 cercariae of *S. haematobium* alone (five animals); received intramuscular injections of 5 mg/kg bw (two animals) or 50 mg/kg bw (three animals) NBHBA per week up to the end of the experiment; or were infected with *S. haematobium* and administered 5 mg/kg bw NBHBA per week throughout the experiment (10 animals). All surviving animals were killed after 2.5 years. No urinary bladder tumour was found in animals receiving either *S. haematobium* or NBHBA alone, but three of the baboons receiving the combined treatment had adenomatous lesions of the urinary bladder described by the authors as 'early or latent adenocarcinomas' and a fourth had a papillary carcinoma. Three baboons had papillary growths in the ureter (Hicks *et al.*, 1980; Hicks, 1982). [The Working Group had difficulty in interpreting some of the diagnostic terms used in these reports.]

3.3 Infection with *Schistosoma mansoni* alone

3.3.1 *Mouse*

Groups of eight male C3H mice, three months old, were injected subcutaneously with one, six or 10–16 lyophilized, immature worms of *S. mansoni* and were examined for palpable tumours at the injection site every two weeks until termination of the experiment at 21 months. No tumours were found at the injection site. The numbers of survivors were

19/24 at 12 months, 11/24 at 18 months and 9/24 at termination. Of the nine mice killed at termination, three had hepatomas and one had a single pulmonary tumour. The authors reported that the frequency of pulmonary and liver tumours in this strain of mice was similar to that in historical controls (Shimkin *et al.*, 1955). [The Working Group noted that an infectious agent was not employed.]

In several combination experiments in mice (Domingo *et al.*, 1967; Haese *et al.*, 1973; Haese & Bueding, 1976; Bulay *et al.*, 1977; El-Aaser *et al.*, 1978; Kakizoe, 1985) in which control groups of untreated mice or mice infected with *S. mansoni* only were used, no increase in the frequency of liver tumours was reported. Some of the experiments lasted less than 50 weeks (see section 3.4).

As part of an experiment to study the carcinogenic potential of hycanthone, groups of female Swiss-Webster mice, four weeks of age, were infected by intraperitoneal injection with 40 or 80 cercariae of *S. mansoni*. Eighteen months later, the incidences of livers with nodules were 15/60 [$p < 0.001$; Fisher exact test] in the group given 40 cercariae and 1/49 [not significant] in that given 80 cercariae. No nodule was found in uninfected paired groups of 61 and 54 animals (Yarinsky *et al.*, 1974). [The Working Group noted that histological examination was not performed.]

3.3.2 *Mastomys natalensis*

A group of 200 *Mastomys natalensis*, about three weeks of age, were injected intraperitoneally with 100 *S. mansoni* cercariae and maintained until death (up to 2.5 years). Infection was confirmed by examination of faeces for ova during life and examination of liver, gut and mesentery for ova and adult worms after death. At the end of the experiment, 106 animals with evidence of infection were available for evaluation. The incidence of adenocarcinomas of the glandular stomach (23/106) did not differ significantly from that expected in controls (~20%). [The common stomach tumours in *M. natalensis* were then described as adenocarcinoma but are now recognized as carcinoids.] In contrast, hepatomas were observed in 22 infected animals; such tumours had not been observed in several hundred historical controls. Two animals also developed reticulum-cell sarcomas of the ileum and colon, respectively, associated with schistosomal granulomas (Oettlé *et al.*, 1959).

3.3.3 *Hamster*

Groups of 35 male and 35 female Syrian golden hamsters were infected by intraperitoneal injection of 15 cercariae of *S. mansoni*. No increase in tumour incidence was observed over that in uninfected hamsters within 73 weeks (Bulay *et al.*, 1977). [The Working Group noted the lack of verification of infection.]

3.3.4 *Nonhuman primate*

One case report of a hepatocellular carcinoma in a 12-year-old female chimpanzee (*Pan troglodytes*) has been published. The animal had been captured in the wild in Sierra Leone when two years of age and had no hepatitis B surface antigen, no antibodies to hepatitis B surface or core antigens and no viral RNA of hepatitis C on arrival at the laboratory, although granulomatous inflammation was seen. After 10 years in captivity, during an

intervention before the start of a study of hepatitis, a firm white nodule was discovered in the liver which, upon histological examination, was found to be a well-differentiated hepatocellular carcinoma. No cirrhosis was present, but a severe granulomatous inflammatory reaction was apparent, with remnants of schistosomal egg capsules. On the basis of morphological examination, the eggs were considered to be *S. mansoni* (Abe *et al.*, 1993).

3.4 Infection with *Schistosoma mansoni* in combination with administration of known carcinogens

3.4.1 *2-Amino-5-azotoluene*

Mouse: A total of 410 female CBA mice, two months of age, were divided into four groups as follows: 80 untreated, uninfected animals, which served as controls; 95 mice that each received subcutaneous injections of 10 mg 2-amino-5-azotoluene in glycerol once a month for nine months; 100 mice that received a single subcutaneous injection of 30 cercariae of *S. mansoni*; and 135 mice that were infected with *S. mansoni* and received 2-amino-5-azotoluene eight weeks later. Between 24 and 52 weeks, six animals from each group were examined periodically for pathological changes in the liver; the remaining animals were maintained until death and were examined for gross liver tumours. At 24 weeks after the beginning of the study, the numbers of animals alive in the four groups were 69/80, 80/95, 31/100 and 35/135, respectively. The authors noted that the high mortality in infected animals was due to the infection. No hepatoma was observed in control animals or in those infected with *S. mansoni* alone. At 52 weeks of age, the incidence of hepatomas was 1/80 in the group treated with 2-amino-5-azotoluene alone and 13/35 in the group given the combined treatment [$p < 0.001$; Fisher exact test] (Domingo *et al.*, 1967; Liu *et al.*, 1969).

3.4.2 *2-Naphthylamine and 2-acetylaminofluorene*

Mouse: Groups of female Swiss albino mice, six to eight weeks of age, were divided at random into the following groups: one group of 45 mice served as untreated controls; one group of 46 mice was infected with *S. mansoni* by immersion [technique unspecified] for 1 h in water containing 20–30 cercariae per millilitre; one group of 20 mice received 1% 2-naphthylamine in the diet; a group of 20 mice received 0.06% 2-AAF in the diet; one group of 17 mice was infected with *S. mansoni* and treated with 1% 2-naphthylamine; and a further group of 22 mice was infected with *S. mansoni* and treated with 0.06% 2-AAF. Administration of the carcinogens was terminated after 30 weeks owing to severe toxicity. The other experimental groups were continued up to 70 weeks. No liver or bladder tumour was observed in any of the groups. All mice infected with *S. mansoni* showed granulomatous areas in the portal tracts of the liver and had ova in the faeces (El-Aaser *et al.*, 1978).

A total of 109 female ddY mice, four weeks of age, were divided into three groups: 45 mice received an intraperitoneal injection of 20 *S. mansoni* cercariae and four weeks later were fed a diet containing 0.03% 2-AAF; 32 mice were infected with *S. mansoni* and fed normal diets; and 32 uninfected mice were fed normal diet for four weeks and subsequently fed a diet containing 0.03% 2-AAF. A number of animals from each group were killed every 10 weeks for interim examination. The experiment was terminated after 40 weeks. No liver

tumour was found in the group infected with *S. mansoni* only. In the group fed 2-AAF only, the incidence of hyperplastic nodules in the liver was 2/32 (6.3%) at 40 weeks. In the group that was both infected and fed 2-AAF, the incidence of hyperplastic nodules was 9/45 (20%) [$p = 0.005$; Fisher exact test]. Hepatocellular carcinomas were found in 12/45 mice in the combined treatment group at weeks 29–40 and 0/32 in the group infected with *S. mansoni* [$p < 0.001$; Fisher exact test] (Kakizoe, 1985).

3.5 Infection with *Schistosoma mansoni* in combination with administration of compounds used or evaluated in the past as antischistosomal agents

A variety of studies were undertaken to determine the effects of hycanthone (Haese *et al.*, 1973; Yarinsky *et al.*, 1974; Haese & Bueding, 1976), niridazole (Bulay *et al.*, 1977) and SQ 18506 (Haese *et al.*, 1973; Dunsford *et al.*, 1984) on tumour induction in uninfected and *S. mansoni*-infected mice and hamsters. These chemicals were used in the past (hycanthone and niridazole) or evaluated for possible use (SQ 18506) as antischistosomal agents; none are currently in use clinically. The agents were studied by various methods and schedules of administration, and various non-tumour and tumour end-points were evaluated, including hyperplastic nodules of the liver, hepatomas, tumours of the stomach and other tumours. Both higher and lower tumour incidences were found with combined treatment than in animals only infected with *S. mansoni*. The lower tumour incidences were presumably due to lowering or elimination of infection. [The Working Group noted that hycanthone was previously categorized in Group 3 and niridazole in Group 2B (IARC, 1987).]

3.6 Infection with *Schistosoma japonicum* alone

Mouse: A group of 395 female SPF ddY mice, four weeks of age, were exposed after anaesthesia with phenobarbital to five or six cercariae of *S. japonicum* on the shaved abdomen; 163 were found to be infected 8–10 weeks after exposure, as shown by the presence of eggs in the faeces. More than half of the infected animals had died within 30 weeks after exposure, and 70 survived to the end of the experiment (50 weeks). Of a control group of 61 females undergoing anaesthesia only, 60 survived to the end of the experiment. Upon autopsy, 9/70 infected mice showed no presence of eggs in the liver or intestine and were excluded from the analysis. Of the 61 remaining treated animals, 48 were found to have hepatomas, whereas none were found in the surviving controls [$p < 0.001$; Fisher exact test] (Amano & Oshima, 1988).

3.7 Infection with *Schistosoma japonicum* in combination with administration of known carcinogens

3.7.1 *Dimethylaminoazobenzene*

Mouse: Three groups of mice [initial numbers, sex and age unspecified] were either infected with *S. japonicum* and received no further treatment; were uninfected and fed a diet containing 20 ml 3% dimethylaminoazobenzene in corn oil mixed with 1 kg of rice powder; or were infected with *S. japonicum* and, 60 days later, fed the diet containing dimethylamino-

azobenzene. Groups of mice were killed at various intervals up to 150 days. The authors reported that the mice that received the combined treatment developed severe liver cirrhosis and had faster hepatic cancer formation than the uninfected, carcinogen-treated mice (Shigefuku, 1943). [The Working Group noted the limited reporting of this early study].

3.7.2 *2-Acetylaminofluorene*

Mouse: Female ddY mice, four weeks of age, were divided at random into two groups. The first group (77 animals) was infected by immersion of the tail in water containing 40 *S. japonicum* cercariae and four weeks later were fed a diet containing 0.03% 2-AAF for 40 weeks; the second group (86 animals) was fed basal diet followed four weeks later by a diet containing 0.03% 2-AAF for 40 weeks. Interim killings of animals were made between weeks 9 and 40 of 2-AAF administration. The first liver tumours were observed 16 weeks after administration of 2-AAF in the infected group and at 37 weeks in the uninfected group. At 40 weeks, the incidence of liver tumours was 24/77 in carcinogen-treated, infected mice and 6/86 in carcinogen-treated, uninfected mice ($p < 0.005$, χ^2 test). The tumour types in the two groups, respectively, were: hyperplastic type 1 nodules, 6 and 4; hyperplastic type 2 nodules, 10 and 2 ($p < 0.01$, χ^2 test); and hepatocellular carcinomas, 8 and 0 ($p < 0.005$, χ^2 test) (Miyasato, 1984).

4. Other Data Relevant for Evaluation of Carcinogenicity and its Mechanisms

4.1 Pathology of infection

4.1.1 *Humans*

(*a*) *Schistosoma haematobium*

Many of the most severe pathological manifestations of schistosomiasis are due to a large extent to a physical and immunological response of the host to the eggs (Parra *et al.*, 1991). A periovular area of granulomas surrounded by an exudative cellular reaction consisting of many polymorphonuclear leukocytes, lymphocytes and eosinophilic cells is found to occur in most granulomatous areas (Nawar *et al.*, 1992; Lukacs *et al.*, 1993).

Clinical and pathological evidence for 'early stage of infection' (haematuria and dysuria) is seen in the majority of infected children and young adults (King *et al.*, 1988). In contrast, 'late stage of infection' may be less symptomatic but associated with structural urinary tract diseases. Asymptomatic infection may still be associated with urinary tract lesions (Abdel-Salam & Ehsan, 1978).

da Silva Lopes (1984) reported a pathological study of 210 malignant tumours (206 carcinomas) of the bladder in Luanda, Angola. Of the 164 carcinomas associated with schistosomiasis, 122 were of the 'spinocellular' type, 15 were 'urothelial', 13 were 'urothelial plus epidermoid metaplasia', 8 were adenocarcinomas and 16 were undifferentiated carcinomas. Of the 42 carcinomas not associated with infection, 30 were 'urothelial', 6 were 'urothelial plus epidermoid metaplasia', 3 were 'spinocellular' and 3 were undifferentiated carcinomas.

(i) *Early stage of infection*

The most significant pathophysiological disease sequelae of the early stage of *S. haematobium* disease occur in the ureters and urinary bladder. Eggs are deposited in particularly large numbers at the lower ends of the ureters. Ureteric lesions result in anatomical or functional stenosis, leading to hydroureters and hydronephrosis. At the site of egg deposition in tissues, circumoval granulomas, fibrosis and muscular hypertrophy may be demonstrated histologically. The same pattern of tissue involvement is seen in the urinary bladder (Smith *et al.*, 1974).

Two major autopsy studies—one in Ibadan, Nigeria (Edington *et al.*, 1970) and the other in Egypt (Smith *et al.*, 1974)—contributed significantly to our appreciation of the pathological changes in schistosomiasis caused by *S. haemotobium*. Edington *et al.* (1970) studied 673 unselected cadavers in Nigeria and found *S. haematobium* in 20%; 183 of the autopsies were performed on individuals under 19 years of age. In Egypt, Smith *et al.* (1974) examined specimens taken at 190 consecutive autopsies and found evidence of *S. haematobium* infection in 117 (61.6%).

The morphological findings in early active *S. haematobium* disease comprise polypoid granulomatous lesions surrounding the parasite eggs. In the urinary bladder, the pathological manifestations are polyposis and/or ulceration. *S. haematobium*-induced bladder polyps consist of large inflammatory masses containing schistosome eggs. The deposition of eggs may be apicocentric, basocentric or diffuse. Apicocentric ova deposition usually occurs at the apex and dome of the urinary bladder, whereas basocentric deposition occurs predominantly in the trigone and lower posterior wall (Christie *et al.*, 1986b). Bladder polyposis is responsible for the haematuria seen in the early stages of infection and in obstructive disease. The other major morphological lesion is bladder ulceration, which may be due to polyp sloughing. Histological examination of bladder tissue in the early stage of infection demonstrates hyperaemia, granulomas around nests of schistosome eggs and early fibrosis and hypertrophy of muscle. Urethelial hyperplasia, metaplasia and dysplasia were significant in all stages of the disease in the series of Smith *et al.* (1974), hyperplasia occurring in 38% of autopsied cases and 21% uninfected controls, metaplasia in 31.6% cases and 11.5% controls and dysplasia in 27.2% cases and 8.5% controls.

(ii) *Late stage of infection*

The change from early-stage to late-stage schistosomiasis caused by *S. haematobium* occurs with age, decrease in parasite load (as determined by urinary egg excretion) and diminished manifestations of acute inflammatory disease, e.g. haematuria. Morphologically, urinary bladder disease in late-stage infection manifests as schistosomal ulcers or sandy patches (Smith *et al.*, 1974). Chronic schistosome-related bladder ulcers usually occur in individuals with previous heavy infection. They are located mainly in the posterior part of the bladder. Sandy patches occur late in infection, most frequently in the trigone area and are covered by irregularly thickened or atrophic mucosa. Histologically, old granulomas may be found in the submucosa and muscularis surrounding disintegrating or calcified ova. In many instances, fibrosis and scanty round-cell infiltration may be seen. Differences between the early and late stages of infection are summarized in Table 8. The eggs of *S. haematobium* tend to calcify and to remain in tissues longer than those of *S. mansoni* (Cheever *et al.*, 1978).

Table 8. Differences between early and late stages of infection with *S. haematobium*

Feature	Early stage of infection	Late stage of infection
Adult worm pairs	Commonly present	Commonly absent
Oviposition	Commonly present	Commonly absent
Urinary egg excretion	Commonly present	Commonly absent
Important in transmission	Yes	No
Granulomatous host response	Present	Absent
Polypoid lesions	Present and possibly obstructive	Very rare
Sandy patches	Present in late active disease	Present and possibly obstructive
Schistosomal obstructive uropathy	Due to obstructive inflammatory polyps	Due to sandy patches obliterating ureteral muscle
Schistosomal ulceration	Uncommon	Frequent
Treatment	Antischistosomal chemotherapy	Surgical repair

Adapted from Smith & Christie (1986)

The concordance of lesions of chronic infection with those of urethelial cancer has been known for over a century. In a series of 1095 patients with urinary bladder cancer in Egypt, *S. haematobium* eggs were found in 82.4% of cases (El-Bolkainy *et al.*, 1981). Well-differentiated squamous-cell carcinomas of the bladder were seen predominantly in patients with eggs and at an earlier mean age than transitional-cell carcinomas. The morphological changes in the urinary bladder associated with the late stage of infection included a spectrum of hyperplasia, squamous metaplasia, dysplastic changes and predominance of squamous-cell carcinoma. Of the 798 squamous-cell carcinomas, 691 occurred in *S. haematobium*-positive samples and 107 in patients with no eggs. Of the 148 cases of transitional-cell carcinoma, 103 were in patients with eggs and 45 in those without.

Similarly, urethelial hyperplasia and squamous metaplasia have been associated with urinary schistosomiasis. Squamous-cell metaplasia of the bladder occurs at increased frequency in schistosomiasis patients and in young people in populations at high risk of squamous-cell carcinoma (Khafagy *et al.*, 1972). Although granulomas occur in both the ureter and bladder, carcinomas occur predominantly in the bladder. In 30 patients with bladder carcinoma in Egypt, the tissue surrounding the tumours usually contained a higher concentration of *S. haematobium* eggs than other areas in the bladder: The egg burden in tissue surrounding the tumour was almost twice the mean in the remainder of the urinary bladder (Christie *et al.*, 1986a).

Further pathological sequelae of *S. haematobium* infection can be seen almost anywhere in the body. The infection may also be associated with other clinical conditions, such as bladder calcification, urolithiasis and pyelonephritis. Most of these lesions are thought to be related to the inflammatory and subsequent fibrotic responses that follow egg deposition in tissues (Cheever *et al.*, 1978).

(*b*) *Schistosoma mansoni*

Infection with *S. mansoni* is often asymptomatic. In studies of populations in endemic areas, morbidity due to *S. mansoni*-induced schistosomiasis was found to be associated with

intensity of infection, particularly in the young (Arap Siongok *et al.*, 1976). Older individuals with light or no parasitologically demonstrable infection may also present with chronic sequelae of disease. Other factors, besides the age of the host, that may play an integral role in the pathogenesis of disease include the geographical strain of parasite, the genetic make-up of the host (Abdel-Salam *et al.*, 1986), water contact and other infectious and nutritional changes.

Disease due to schistosomiasis caused by *S. mansoni* may be classified according to the natural history of infection: cercarial invasion and dermatitis, maturing worms and acute schistosomiasis (Katayama fever) or established infection and intestinal–hepatic disease. Disease may also be classified into mild and severe forms according to its association with intensity of infection and the immunopathological responses of the host.

(i) *Early stage of infection*

Clinical and pathological changes during the acute phase of infection may manifest as cercarial dermatitis, Katayama fever and established intestinal, hepatosplenic and other features of morbidity. Cercarial dermatitis is a sensitization due to invasion of the skin by cercariae. Morphologically, the lesions are maculopapular eruptions, with oedema and round-cell and eosinophil infiltration. In most circumstances, cercarial dermatitis is self-limiting. Early-stage schistosomiasis may occur four to eight weeks after exposure, usually in infected individuals with a high worm load. Early infection is usually found in individuals with no prior exposure to schistosomes. Disease manifestations resemble those of serum-sickness syndrome and are characterized by hepatosplenomegaly, fever, lymphadenopathy and peripheral blood eosinophilia. The pathological features are nonspecific. Katayama syndrome is self-limiting in most circumstances; severe cases may be associated with heavy infection and may be fatal.

The morphological features that characterize acute established infection are related to the severe inflammatory response around mature eggs in tissues. Large periovular granulomas with prominent necrotic–exudative features are seen. Microscopically, mature eggs are surrounded by round-cell and eosinophilic infiltrations with necrosis and the development of fibrosis (Cheever *et al.*, 1978).

(ii) *Late stage of infection*

Hepatic disease is the best characterized feature of the late stage of *S. mansoni* infection (Kamel *et al.*, 1978). Granulomas around schistosome eggs first cause obstruction of the finest portal radicles at the periphery of the liver. With progression of inflammation, increased intrahepatic portal pressure occurs, leading to the opening up of fine collaterals around the main portal branches. Simultaneously, fibrosis follows inflammation, and the classical clay-pipe-stem fibrosis becomes the dominant feature, with its haemodynamic sequelae.

Colonic inflammatory pseudopolyposis [the Working Group noted that these lesions are not neoplastic] was described in 30 men in Egypt who were infected with *S. mansoni*, *S. haematobium* or both. Most of the pseudopolyps occurred in the rectosigmoid colon. Microscopically, the lesions contain mononuclear cells and eosinophils; the colonic glands show proliferation and distortion but no adenomatous change. Ulcers are frequently reported on the surface of colonic polyps (Smith *et al.*, 1977).

(*c*) *Schistosoma japonicum*

The pattern of infection and disease due to *S. japonicum* infection in general follows closely the sequence of events in schistosomiasis caused by *S. mansoni*: swimmers' itch (cercarial dermatitis), Katayama fever and progression of the disease, leading to established infection (Domingo *et al.*, 1980; Warren *et al.*, 1983). The major differentiating feature is the morphology of the host granulomatous response around the eggs. Granulomas around *S. japonicum* eggs usually occur around nests rather than isolated eggs. In early-stage infection, the lesions look like abscesses with central necrosis. Early-stage acute granulomas consist of eosinophils, lymphocytes and a few histiocytes. At the late stage, histiocytes become more prominent, with the formation of multinucleated giant cells phagocytosing pieces of egg shell. The end result is a fibrotic lesion with a certain degree of hyaline degeneration (Kurniawan *et al.*, 1976).

Chen *et al.* (1980) compared 289 cases of colorectal carcinoma associated with schistosomiasis with 165 cases not associated with the parasite in China. Well-differentiated adenocarcinomas accounted for 91.6% of the malignant tumours in patients with schistosomiasis and 69.1% in patients without schistosomiasis. Benign adenomatous and papillary polyps were found in 6.4% of patients with schistosomiasis and in 29% of patients without schistosomiasis. The same group of investigators (Chen *et al.*, 1981) conducted a retrospective review of specimens taken by colectomy from 60 patients with schistosomiasis. They described 36 lesions as dysplasia, which occurred in the flat mucosa, in pseudopolyps or in regenerative epithelium at the edges of ulcers. The incidence of dysplasia in the colon was not reported for people not infected with schistosomes. Another study from China (Yu *et al.*, 1991) included the results of mass screening for colorectal carcinoma, which led to the taking of 754 biopsy specimens from patients over 30; 320 polyps were studied histologically and were found to be distributed about equally between fibrous, mixed and epithelial polyps. Sialomucins and carcinoembryonic antigens were found more frequently in epithelial than in other types of polyps. [The Working Group noted that the terminology used is confusing and the relevance to carcinogenicity is uncertain.]

4.1.2 *Experimental systems*

(*a*) *Schistosoma haematobium*

Repeated attempts have been made to infect several species of experimental animals with *S. haematobium* (Kuntz *et al.*, 1972), but no satisfactory model that reproduces infection and disease as it occurs in humans has yet been described. Webbe *et al.* (1974) demonstrated that infection of baboons (*Papio anubis*) results in passing of viable eggs in urine and faeces. Macroscopic bladder lesions have been reported to vary from pinhead discoloured elevation of mucosa to gross polypoid masses. Eggs have been seen scattered throughout subepithelial layers and surrounded by a predominantly eosinophilic infiltrate. No evidence of malignant transformation was reported. Similar lesions were seen in the ureters. The pathophysiological sequelae included distorted ureters, hydronephrosis and ureteric calculi.

(*b*) *Schistosoma mansoni*

Animal models have made it possible to study the pathogenesis of granuloma formation and fibrosis due to this species of schistosome. For example, *S. mansoni* infection in mice

results in granuloma formation and disease in the intestines and liver. It was estimated that 63% of ova produced by the schistosome in the porto-mesenteric system were retained in the murine host. Egg deposition was followed by a delayed hypersensitivity granulomatous response which is central to the pathogenesis of disease in the intestine and liver (Warren, 1973). Hepatic egg granulomas are located in all the presinusoidal areas and result in hepatomegaly and destruction of portal blood flow. The haemodynamic consequences lead to portal hypertension, splenomegaly and oesophageal varices, which may bleed. Granulomas are finally replaced by fibrous tissue in the liver, resulting in a unique form of liver fibrosis (Olds *et al.*, 1989) in which hepatic parenchyma and perfusion are retained for a long time.

The regulation of granuloma formation has been carefully studied in the murine model (Warren, 1973; Henderson *et al.*, 1991, 1992). Parasite ova lodge in the small pulmonary vessels, and the host reacts to their presence by forming delayed hypersensitivity granulomas. These isolated lesions can be studied with respect to their composition, the basis of their induction and regulation and immunological reactions. The granuloma is made of lymphocytes, mononuclear phagocytes and eosinophils, but this rich cellular infiltrate is later replaced by scar tissue, with a marked reduction in cellularity. Several cytokines have been shown to be involved in the induction of granuloma, including interleukins 2 and 4 and interferon-γ (Henderson *et al.*, 1991, 1992; Lukacs & Boros, 1993). Granulomas that form in animals with chronic infection are smaller than those seen during the acute phase. This down-regulation or modulation of granuloma formation has been shown to be immunologically regulated and to be dependent on the interaction of Th1 and Th2 subsets of lymphocytes (Lukacs & Boros, 1993).

In baboons and chimpanzees infected with *S. mansoni*, the disease sequence closely resembles the features seen in infected humans (Warren, 1973).

(*c*) *Schistosoma japonicum*

Several species of subhuman primates and rodents exhibit a host–parasite relationship similar to *S. japonicum* infection in humans (Cheever, 1985).

4.2 Other observations relevant to the interpretation of carcinogenicity and mechanisms of carcinogenesis

4.2.1 *Humans*

Numerous explanations have been offered for the proposed association between schistosomiasis and human cancers. Generally, these can be categorized as involving: exogenous and endogenous agents which induce DNA damage (Abdel-Tawab *et al.*, 1968a,b; Fripp & Kean, 1980; Hicks, 1982; Gentile, 1991) or possible tumour promoting activity (Ishii *et al.*, 1989); altered host metabolism (El-Aaser *et al.*, 1982; Gentile, 1985; Gentile *et al.*, 1985); pathological changes leading to increased cell proliferation (Ishak *et al.*, 1967; Brand & Brand, 1980a,b; Rosin *et al.*, 1994); and immune reactions (Raziuddin *et al.*, 1991, 1992, 1993; Gentile & Gentile, 1994).

Endogenous agents may be introduced into schistosome-infected organs in several ways. For example, quantitatively altered tryptophan metabolism in *S. haematobium*-infected

patients results in higher concentrations of certain metabolites (e.g. indican, anthranilic acid glucuronide, 3-hydroxyanthranilic acid, L-kynurenine, 3-hydroxy-L-kynurenine and acetyl-L-kynurenine) in pooled urine (Abdel-Tawab *et al.*, 1966a, 1968b). Some of these metabolites have been reported to be carcinogenic to the urinary bladder in implantation experiments (Allen *et al.*, 1957; Bryan *et al.*, 1964; Bryan, 1969; Röhl *et al.*, 1969).

Other endogenous agents may be involved in secondary bacterial infection. Bacterial urinary tract infections such as those that occur subsequent to the late sequelae of *S. haematobium* infection may play an intermediary role in the genesis of squamous-cell carcinoma. Secondary bacterial infection of *Schistosoma*-infected bladders is a well-documented event (Laughlin *et al.*, 1978; Hill, 1979; El-Aaser *et al.*, 1982; Hicks *et al.*, 1982).

Nitrosamines formed by bacterial catalysis (or via urinary phenol catalysis) of the nitrosation of secondary amines with nitrites have been detected in urinary bladders from *S. haematobium*-infected patients; they may be carcinogenic to bladder mucosa (Hicks *et al.*, 1977, 1978, 1982; Tricker *et al.*, 1989, 1991). Mostafa *et al.* (1994) also demonstrated the presence of nitrates and nitrites in the saliva and increased concentrations of *N*-nitroso compounds in the urine of *S. mansoni*- or *S. haematobium*-infected people who were not on controlled diets. The etiological significance of these findings is, however, unclear in the light of the finding that urine from schistosomiasis patients is not mutagenic (Everson *et al.*, 1983).

Nitrosamines have been detected in the urine of paraplegic patients with urinary tract infections due to urinary stasis (Hicks *et al.*, 1977, 1978).

In a US case–control study in which 2982 urinary bladder cancer patients (97% with transitional-cell carcinomas) were compared with 5782 controls (Kantor *et al.*, 1984), odds ratios of 1.5 (95% CI, 1.3–1.8) in males and 1.2 (0.9–1.5) in females reflect an association with one or two past urinary tract infections, and odds ratios of 2.0 (1.6–2.6) in males and 2.1 (1.6–2.7) in females reflect an association with three or more such infections. For the 39 patients with squamous-cell carcinomas, odds ratios of 1.9 (0.7–4.8) for having had one or two infections and 4.8 (1.9–11.5) for three or more infections were found for the two sexes combined. Adjustments were made for race, age, smoking and, for squamous-cell cancer, sex.

On follow-up of 6744 British paraplegic patients (who are subject to frequent urinary tract infections), 25 urinary bladder cancers were identified (El Masri & Fellows, 1981). On the basis of information for an otherwise comparable population, 1.6% of these would have been expected to be of squamous origin, whereas 44% actually were (estimated relative risk, 49; 95% CI, 20–119). In Uganda, squamous-cell bladder cancers are commonly seen in the absence of *S. haematobium* infection but in the presence of other urinary tract abnormalities (Anthony, 1974).

One of the prevalent theories for the association between schistosomal infection and cancer is that elevated levels of the enzyme β-glucuronidase in the host could increase the release of carcinogenic metabolites from their glucuronides. No data are available at present to confirm this association, although schistosome-infected humans are known to have elevated β-glucuronidase activity in urine (Fripp, 1960; Abdul-Fadl & Metwalli, 1963; Fripp, 1965; Abdel-Tawab *et al.*, 1966b, 1968a; Norden & Gefland, 1972; El-Sewedy *et al.*, 1978;

El-Zoghby *et al.*, 1978; El-Aaser *et al.*, 1979). The cause of the increase in β-glucuronidase activity in individuals suffering from schistosomiasis is unknown.

Several studies provide evidence for genetic damage in schistosomiasis patients. Sister chromatid exchange and micronucleus frequencies are increased slightly in peripheral blood lymphocytes harvested from schistosomiasis patients (Shubber, 1987; Anwar, 1994), and micronuclei were more frequent in urothelial cells from chronic schistosomiasis patients than in controls (Rosin & Anwar, 1992). The mean frequency of micronuclei was reduced significantly after treatment with praziquantel, which may indicate that infection is involved in chromosomal breakage in the urothelium (Anwar & Rosin, 1993).

No mutation was detected at codon 12 of the H-*ras* oncogene in nine squamous-cell carcinomas associated with schistosomiasis (Fujita *et al.*, 1987). Mutations of the *p53* tumour suppressor gene were detected in six of seven squamous-cell carcinomas associated with *S. haematobium*; no specific pattern of mutation emerged, in contrast to the pattern seen in transitional-cell carcinomas related to tobacco smoking (Habuchi *et al.*, 1993). O^6-Methyldeoxyguanosine was detected in DNA from 44 of 46 Egyptian samples of bladder tissue, 38 from tumour tissue and eight from uninvolved bladder mucosa, and in 4 of 12 normal samples of bladder of European origin (Badawi *et al.*, 1992a).

4.2.2 *Experimental systems*

(*a*) *Schistosoma haematobium*

Capuchin monkeys (*Cebus apella*) and African baboons (*Papio cynocephalus*) were exposed to 500–3000 cercariae, which produced active schistosomiasis and associated pathological manifestations (Brown *et al.*, 1976). Analysis of urine samples collected when the infection was declared (5–8 months after infection) showed accumulation of high levels of 3-hydroxykynurenine and 3-hydroxyanthranilic acid, indicating altered tryptophan metabolism in the host.

Syrian hamsters infected with 200 cercariae of *S. haematobium* had elevated β-glucuronidase activity, and their livers had reduced competence to metabolize the urinary bladder carcinogen 3,3′-dichlorobenzidine. The mutagenic potential of this chemical to bacteria was significantly enhanced in the presence of urine from the infected animals, liver enzymes and β-glucuronidase (Gentile *et al.*, 1985).

(*b*) *Schistosoma mansoni*

The modified metabolic profiles of xenobiotics in parasite-infested hosts have been studied extensively (for a general review of altered xenobiotic metabolism in parasitic diseases, see Tekwani *et al.*, 1988). In most of these studies, mice were used as hosts and exposed to 100–200 cercariae. The xenobiotics studied include lindane (Mostafa *et al.*, 1984), *N*-nitrosodimethylamine (Mostafa *et al.*, 1984), 2-acetylaminofluorene (Siwela *et al.*, 1990) and aflatoxin B_1 (Daneshmend, 1984). The evidence suggests that alterations in the carcinogen metabolizing capacities of the liver of mice infected with *S. mansoni* lead to a decreased capability to process xenobiotics. Infected hosts also have enhanced enzymatic activity for some other enzymes, such as β-glucuronidase. O^6-Methyldeoxyguanosine was found in DNA of the liver (but not of other organs) of *S. mansoni*-infected mice, again implying an abnormal metabolic profile in infected livers (Badawi *et al.*, 1992a,b,1993).

(*c*) *Schistosoma japonicum*

Sequence homologies to the *env* gene of mouse ecotropic and xenotropic retroviruses were detected in the DNA of adult worms (Tanaka *et al.*, 1989). Iwamura *et al.* (1991) made similar findings in adult worms and in DNA isolated from eggs. Host (mouse)-related DNA sequences were identified in the subtegumental layer and inner tissues of adult *S. japonicum* by in-situ hybridization with ^{32}P-labelled probes (Irie & Iwamura, 1993).

Reduced levels of cytochrome P450 have frequently been reported in infected animals (see Tekwani *et al.*, 1988, for a review). These results were confirmed in mice infected with *S. japonicum* (Matsuoka *et al.*, 1989), and the same authors demonstrated that liver homogenate from *S. japonicum*-infected mice had a reduced mutagen activating potential for 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). Hepatic fractions from infected mice had a lower mutagen-activating capacity than hepatic fractions from uninfected mice when Trp-P-2 was used as the substrate (Arimoto *et al.*, 1992). A similar observation was made with aflatoxin B_1: microsomes from infected mice were less effective at producing ^{3}H-AFB_1 covalent binding than microsomes from uninfected animals (Hasler *et al.*, 1986).

S. japonicum-infected mice, however, maintain higher levels of serum Trp-P-2 given intravenously than uninfected mice treated in the same way, suggesting that although infected animals have lowered metabolism increased retention of the mutagen can occur (Aji *et al.*, 1994). This persistence could result in Trp-P-2 complexes with haem *in vivo* (Arimoto *et al.*, 1980; Arimoto & Hayatsu, 1989).

The mutagenicity of the parasite itself was investigated in bacterial bioassays; extracts of neither eggs nor adults were mutagenic to *Salmonella typhimurium* or *Escherichia coli* (Ishii *et al.*, 1989).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Schistosomes are trematode worms that live in the bloodstream of human beings and animals. Three species (*Schistosoma haematobium*, *S. mansoni* and *S. japonicum*) account for the majority of human infections. People are infected by exposure to water containing the infective larvae (cercariae). The worms mature in the veins that drain the bladder (*S. haematobium*) or in the intestine (other species). The adults do not multiply in the body but live there for several years, producing eggs. Some eggs leave the body in the urine or faeces and hatch in water to liberate the miracidium larva, which infects certain types of freshwater snails. Within the snail, the parasites multiply asexually to produce free-swimming cercaria larvae, which infect people by skin penetration. Eggs remaining in the human body are trapped in the tissues, where they elicit hypersensitivity granulomas that cause disease in the urogenital system (*S. haematobium*) or in the liver and intestines (other species).

The diagnosis of infection with *Schistosoma* is based on simple qualitative and quantitative examinations of faeces and urine. *S. haematobium* infection is identified on the basis of a history of haematuria, observation of gross haematuria, detection of haematuria by chemical reagent strips or detection of eggs in urine by microscopy. *S. mansoni* and

S. japonicum infections are identified by the presence of eggs in faeces. All infections can be quantified by egg counts in urine (*S. haematobium*) and faeces (other species). The available immunodiagnostic tests are useful for detecting light infections. Absence of infection can be established with certainty only by use of a combination of diagnostic tests.

Schistosomiasis occurs in at least 74 countries where 600 million people are at risk, of whom over 200 million are infected. The distribution of infection corresponds to the distribution of the snail hosts. Within endemic areas, transmission may be focal and can be localized to specific water sources. The intensity and frequency of exposure to contaminated freshwater determine the occurrence of the heavy infection that leads to disease. Prevalence and intensity of infection are usually correlated in endemic areas and especially in children. Sex differences in intensity of infection have been linked to differences in exposure. Death may be caused by urinary tract disease in *S. haematobium* infection and by portal hypertension in *S. mansoni* and *S. japonicum* infection.

Infection with *Schistosoma* is not synonymous with clinical disease, and many infections are asymptomatic. The outcome of infection is influenced by genetic factors, the immune response of the host and concomitant infections (e.g. hepatitis). Clinical disease is a sequel of heavy infection. Treatment of all forms of schistosomiasis with safe, effective anti-schistosomal drugs (i) results in a high rate of resolution of infection, (ii) prevents development of disease in people with heavy infection, (iii) arrests progression of existing severe disease and (iv) reverses some disease manifestations, particularly in children. Control of schistosomiasis has been achieved in some countries through combined approaches to intervention, including health education, improved water supplies and sanitation, environmental management, snail control and treatment.

5.2 Human carcinogenicity data

Schistosoma haematobium

A number of studies from Africa have shown that the estimated incidence of urinary bladder cancer is higher in areas with a high prevalence of infection with *S. haematobium* than in areas with a low prevalence. For example, urinary bladder cancer as a proportion of all cancer appears to be 10 times commoner among men in Egypt than among men in Algeria. Several other observations support an association between the occurrence of urinary bladder cancer and *S. haematobium* infection: the estimated incidence of urinary bladder cancer was related to the proportion of cancerous urinary bladder specimens containing *S. haematobium* eggs or egg remnants; the sex ratio of urinary bladder cancer cases varied widely and corresponded to the relative involvement of men and women in agricultural work (a risk factor for *S. haematobium* infection); and squamous-cell cancers of the urinary bladder were proportionately commoner in populations with a high prevalence of infection with *S. hematobium* and a high proportion of urinary bladder cancers showing histological evidence of infection than in areas without these characteristics.

Many cases of urinary bladder cancer have been reported in association with schistosomal infection of the urinary bladder. Other cancers have been reported in association with infection with *S. haematobium* including, particularly, cancer of the cervix.

Seven case–control studies of the association between *S. haematobium* infection and urinary bladder cancer have been reported. *S. haematobium* infection was measured variously by presence of eggs in urine, pelvic X-ray, rectal biopsy, biopsy of the urinary bladder and digestion and centrifugation of urinary bladder tissue. All of the studies were hospital-based and in none was the correspondence between the population giving rise to the cases and that sampled for the controls demonstrated or addressed in the analysis. Possible confounding by age and sex was not considered in four studies. In three of these four studies, the method of measurement of past infection with *S. haematobium* differed between cases and controls. Possible confounding by smoking was considered in only one study. Six of the seven studies showed significant, positive associations between the occurrence of urinary bladder cancer and infection with *S. haematobium*, with estimated relative risks ranging from 2 to 14. Confounding is not likely to explain the strong associations seen in these studies.

Schistosoma mansoni

A number of cases of liver cancer, colorectal cancer, giant follicular lymphoma and some other cancers have been reported in association with *S. mansoni* infection.

Schistosoma japonicum

Mortality from liver cancer and prevalence of infection with *S. japonicum* have been found to be positively correlated in Japan but not consistently so in China. Mortality from and, in one study, incidence of colorectal cancer were strongly, consistently and significantly correlated with various measures of infection with *S. japonicum* in many studies across provinces, counties and communes in China.

In three case–control studies of liver cancer and infection with *S. japonicum* in Japan and China, the estimated relative risks for the association varied from 2 to 10. The relative risk remained elevated in patients who did not have antigens to hepatitis virus. The two studies giving the highest estimated relative risks were hospital-based and did not address the issue of correspondence between the population giving rise to the cases and that sampled for the controls. In one of these studies, possible confounding by age and sex was not controlled for.

In one hospital-based case–control study of gastric cancer in Japan, the estimated relative risk for *S. japonicum* infection, based on the presence of eggs in tissue, was 1.8 and was significant. Possible confounding by age and sex was not controlled for, and the issue of correspondence between the population giving rise to the cases and that sampled for the controls was not addressed.

Three case–control studies of colorectal cancer and infection with *S. japonicum* have been reported from China and Japan. In one, the estimated relative risks for cancer of the colon in association with the presence of eggs in tissue was about 2.5 and was significant. Possible confounding by age, sex, area of residence, smoking and family history of cancer of the colon was controlled for in this study. In the two other studies, the estimated relative risks were 1.2 for colon cancer in both studies and 8.3 for rectal cancer in one study with control for possible confounding by age, sex, place of residence and occupation.

5.3 Animal carcinogenicity data

Infection with *S. haematobium* has been studied in experiments in mice, rats, hamsters, opossums and nonhuman primates. In mice, hamsters and opossums, hyperplasia of the urinary bladder was observed; one tumour of the urinary bladder was reported in an opossum. The studies in rats were inadequate for evaluation. In nonhuman primates, hyperplasia of the urinary bladder and a few lesions described as tumours of the urinary bladder or ureter were reported. *S. haematobium* infection was also studied in animals treated with known urinary bladder carcinogens. Infection with the parasite increased urinary bladder tumour incidence in mice administered 2-acetylaminofluorene and in baboons treated with *N*-nitrosobutyl-4-hydroxybutylamine.

In one experiment with *Mastomys natalensis* infected with *S. mansoni*, an increased incidence of liver tumours was observed. One case report of a hepatocellular carcinoma in a chimpanzee with *S. mansoni* infection has been published. Infection with *S. mansoni* was studied in inadequate experiments in mice and hamsters. An increased incidence of liver tumours was seen in one experiment in mice infected with *S. mansoni* and treated with 2-amino-5-azotoluene and in one experiment in infected mice treated with 2-acetylaminofluorene.

Infection of mice with *S. japonicum* resulted in a significantly increased incidence of liver tumours in one experiment. Infection with *S. japonicum* enhanced the liver tumour incidence in mice treated with 2-acetylaminofluorene in one experiment.

5.4 Other relevant data

S. haematobium induces chronic inflammation of the lower urinary tract, leading to obstruction, squamous metaplasia, urinary retention and secondary bacterial infections.

Carcinomas of the urinary bladder seen in association with *S. haematobium* infection are more frequently of the squamous-cell type than of the transitional-cell type. Some characteristics of *S. haematobium* infections of the urinary tract may be relevant to the genesis of squamous-cell carcinoma of the bladder. Inflammatory changes are seen in the mucosa of the lower urinary tract. Endogenous mutagenic and carcinogenic products are detected in increased concentrations in the urine of people infected with *S. haematobium*. Recurrent bacterial infection of the urinary tract, even in the absence of *S. haematobium* infection, is strongly associated with the appearance of squamous-cell carcinomas of the urinary bladder. In a small series of patients, mutations at the *p53* gene in squamous-cell carcinomas found in association with *S. haematobium* infection were different from those in the transitional-cell malignancies of smokers.

S. mansoni and *S. japonicum* induce fibrosis of the liver and inflammatory lesions of the large bowel. There is some evidence that livers infected with *S. japonicum* (and other species) alter the metabolism of certain carcinogens.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of infection with *Schistosoma haematobium*.

There is *inadequate evidence* in humans for the carcinogenicity of infection with *Schistosoma mansoni*.

There is *limited evidence* in humans for the carcinogenicity of infection with *Schistosoma japonicum*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Schistosoma haematobium*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Schisotosoma mansoni*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Schistosoma japonicum*.

Overall evaluations

Infection with *Schistosoma haematobium* is carcinogenic to humans (Group 1).

Infection with *Schistosoma mansoni* is not classifiable as to its carcinogenicity to humans (Group 3).

Infection with *Schistosoma japonicum* is possibly carcinogenic to humans (Group 2B).

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INFECTION WITH LIVER FLUKES
(*Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis*)

1. Exposure Data

1.1 Structure and biology of liver flukes

1.1.1 *Taxonomy*

Three of the human liver flukes, *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*, are pathologically important food-borne members of the class Trematoda (Beaver *et al.*, 1984). These flukes establish a chronic infection within the smaller intrahepatic bile ducts and occasionally in the pancreas and gall-bladder of humans and other fish-eating mammals.

The life cycle of food-borne trematodes is complex, involving two more intermediate hosts (the first always a snail) and several morphological stages. The consumption of raw or incompletely cooked foods which contain the infective stages is the major risk factor for these infections. As a result, people who enjoy a variety of raw foods often harbour several trematodes (liver and intestinal flukes). Similarities in egg morphology and cross-reactive antigens complicate both parasitological and immunological diagnosis and may confound clinical and epidemiological research on the liver flukes.

Fish-eating mammals, for example dogs, cats, pigs, mink, weasels and civets, may become infected with human liver flukes, and some may act as reservoir hosts (Beaver *et al.*, 1984).

1.1.2 *Structure*

In humans, *Clonorchis* measures 8–15 mm long and 1.5–5 mm wide, while the two *Opisthorchis* species are somewhat smaller—3–12 mm by 1–3 mm. They are covered by a tegument and have an oral sucker at the anterior end and a ventral sucker or acetabulum located posterior at about one-third to one-fifth of the body length. Adult worms are hermaphroditic, with reproductive organs occupying much of the body (Sadun, 1955; Komiya, 1966; Beaver *et al.*, 1984; Rim, 1986).

The morphology of the adult worms of *O. viverrini*, *O. felineus* and *C. sinensis* is similar but can be distinguished at the cercarial stage by the bilateral pattern of excretory flame cells (Vajrasthira *et al.*, 1961; Wykoff *et al.*, 1965). The adults differ in the shape of testicular lobes, their location relative to the ovary and the appearance of vitelline glands (Sadun, 1955; Wykoff *et al.*, 1965). The metacercariae and juvenile worms bear spines.

The yellowish-brown eggs have a distinct operculum, which opens to release the miracidia when the eggs are ingested by an appropriate species of snail. The posterior end of the

egg has a small protuberance, or knob. Eggs average 29 μm long by 15 μm wide for *C. sinensis* (Ditrich *et al.*, 1992), 27 by 15 μm for *O. viverrini* (Sadun, 1955; Kaewkes *et al.*, 1991) and 30 by 11 μm for *O. felineus* (Ditrich *et al.*, 1990), with differences in shape between species.

1.1.3 *Life cycle and biology of the adult worm*

The life cycle of liver flukes is illustrated in Figure 1.

Infection with *Opisthorchis* and *Clonorchis* is acquired through the consumption of raw or undercooked fish containing the infective stage, called metacercaria. The metacercariae leave the cyst in the duodenum and migrate through the ampulla of Vater via the common and extrahepatic bile ducts to the smaller, proximal bile ducts under the surface of the liver, where they mature. Although most adult worms are found in these ducts, in heavy infections they can be found in the extrahepatic bile ducts, pancreatic ducts and, rarely, the gall-bladder (Hou, 1955; Sithithaworn *et al.*, 1991a). Infection is confined to the lumen of the hepato-biliary tract; there is no phase of tissue migration, even when the common bile duct is severed (Sun *et al.*, 1968).

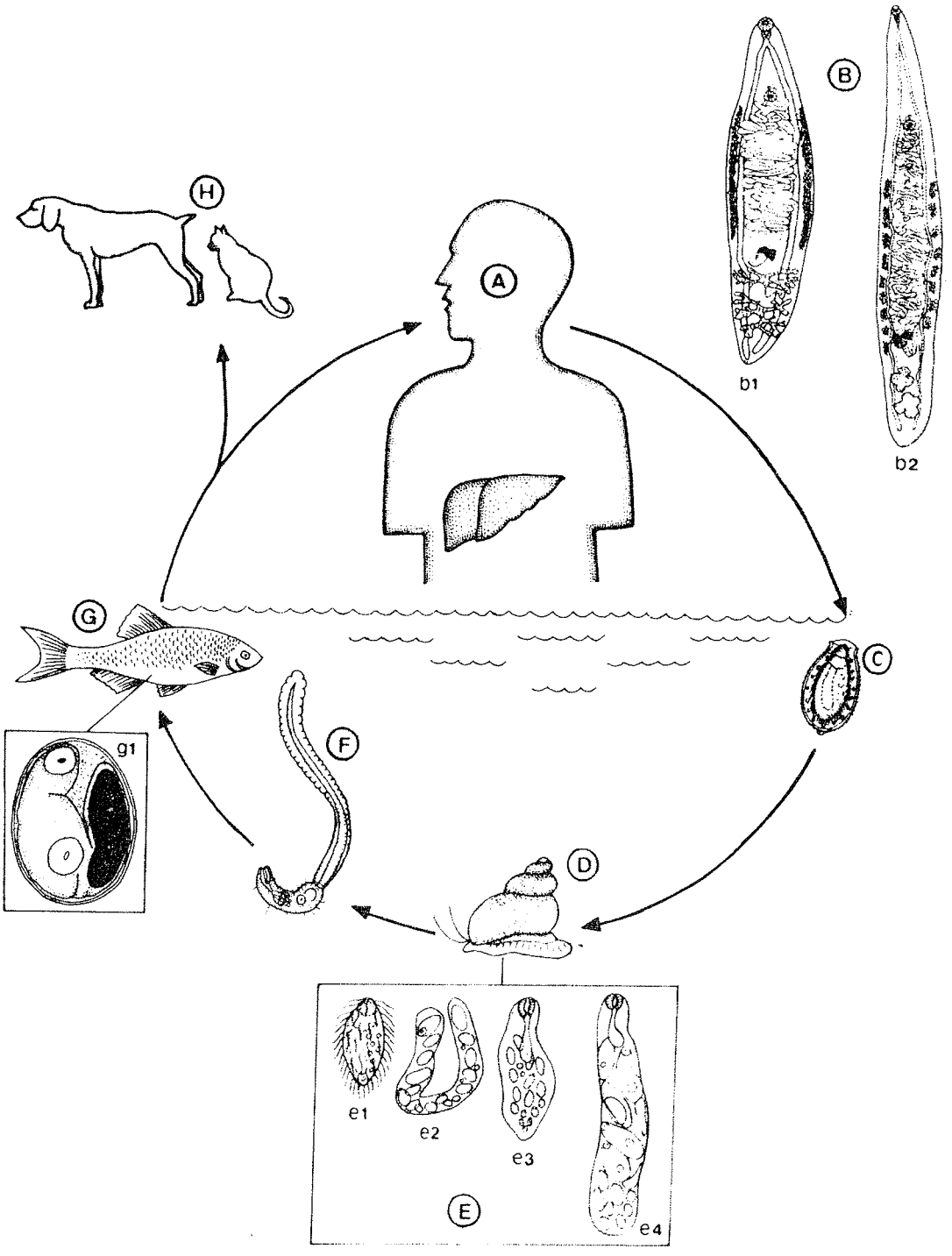
Clonorchis moves up the biliary tract by attaching and detaching its two suckers and extending and contracting its body. Its attachment to the wall of the bile duct may be secured by adherence of the ventral sucker to the biliary epithelium, leaving the oral sucker free for feeding (Hou, 1955).

About one month after the metacercariae have been ingested, adult worms begin producing eggs, which pass down the bile duct and are excreted in the faeces. Eggs can also be found in gall-bladder bile. The average egg output per gram of faeces per adult *Opisthorchis* worm ranges from 15 to 180 (Elkins *et al.*, 1991; Sithithaworn *et al.*, 1991b). Density-dependent decreases in fecundity have been documented, which partially explain the wide variation in estimates between these studies (Ramsay *et al.*, 1989). Estimated fecundity in infected people and animals is generally in the range of 1000–5000 eggs per day (Komiya, 1966; Wykoff & Ariyaprakai, 1966; Rim, 1986). The average lifespan of the worms, inferred from epidemiological data, is about 10 years, while the maximal lifespan in the absence of reinfection may exceed 25 years (Attwood & Chou, 1978; Zhu, 1984).

If the eggs reach a body of freshwater (small ponds, streams and rivers, flooded rice fields and large reservoirs), they are ingested by snails. Asexual reproduction in the snail results in the release daily of thousands of cercariae one to two months after infection of the snail. The free-swimming cercariae penetrate the tissue of fish and encyst, becoming fully infective metacercariae after 21 days. Over 80 species of the Cyprinidae family and some 13 species of other families, and possibly freshwater prawns, can serve as the second intermediate host (Komiya, 1966; Vichasri *et al.*, 1982; Rim, 1986; Joo, 1988).

In any defined freshwater body, only 1–3% of snails may be infected, while up to 100% of fish may contain metacercariae (Vichasri *et al.*, 1982; Rim, 1986; Brockelman *et al.*, 1986). The distribution patterns of metacercariae in fish determine patterns of human exposure in endemic areas. The intensity of liver fluke infection in fish varies from one to hundreds, depending on season, type of water body, species and individual. Transmission is seasonal, as a result of patterns of human faecal contamination, water temperature and snail or fish density.

Figure 1. Life cycle of liver flukes



A: definitive host, human; B: adult liver flukes in bile duct, *Clonorchis sinensis* (b1), *Opisthorchis viverrini* (b2); C: embryonated egg; D: first intermediate host, *Bithynia* sp.; E: intramolluscan stages, miracidium (e1), sporocyst (e2), mother redia (e3), daughter redia (e4); F: cercaria; G: second intemediate host (cyprinoid fish), metacercaria in fish muscle (g1); H: reservoir host, dog and cat

The prevalence of infection in reservoir hosts varies by area and is not closely associated with human infection patterns. In endemic areas, transmission to snails is due mainly to human eating patterns, poor sanitation and high egg excretion (Sadun, 1955; Rim, 1986); the importance of reservoir hosts is limited.

1.1.4 *Immune response to infection*

The existence of protective immune responses to liver fluke infections remains unclear (Sirisinha, 1984). Several experimental studies have suggested that small decreases in the establishment or fecundity of worms can be observed in animals that receive repeated infections, spleen cells or serum from infected donors and immunization with parasite antigens (Flavell *et al.*, 1980; Flavell, 1982; Sirisinha *et al.*, 1983; Sirisinha, 1984; Kwon *et al.*, 1987). Flavell and Flavell (1986) reported that animals deprived of T cells had similar worm burden and egg output to intact animals after an equivalent challenge. Wongratanacheewin *et al.* (1987) reported that *O. viverrini* infection was associated with reduced immunoresponsiveness to red blood cells and mitogens, an effect that was reversed by praziquantel treatment.

In humans, the parasites clearly survive high levels of parasite-specific immunoglobulins G, A and E in both serum and bile (Wongratanacheewin *et al.*, 1988). While experimental studies suggest that parasites may induce immunosuppression, no evidence of suppressed skin test reactivity or reduced responsiveness during infection has been observed in humans (Wongratanacheewin *et al.*, 1988; Haswell-Elkins *et al.*, 1991a). Epidemiological patterns reveal little evidence of, but do not rule out, protective immunity. There appears to be no decline in prevalence of infection among individuals exposed to infection for decades, and rapid rates of reinfection have been reported after treatment in areas of heavy infection (Upatham *et al.*, 1988).

High levels of parasite-specific antibodies have been reported in people with severe hepatobiliary disease and cholangiocarcinoma (Srivatanakul *et al.*, 1990; Haswell-Elkins *et al.*, 1991a). Antibody levels are correlated more closely with clinical indicators of infection, such as gall-bladder size and function, wall abnormalities and ultrasound echogenicity of the portal triad, than is egg count (Haswell-Elkins *et al.*, 1991a; Mairiang *et al.*, 1992).

1.2 Methods for detection of infection

1.2.1 *Qualitative faecal examination for eggs*

Detection of liver fluke infection is most often based on the observation of eggs in faeces. The techniques that have been used, in increasing order of sensitivity, are: direct smear, sedimentation, Kato technique, Stoll's technique and formol–ethyl acetate/ether concentration (FECT) (Viyanant *et al.*, 1983; Feng & Chen, 1985; Zavoikin *et al.*, 1985, 1986; Sithithaworn *et al.*, 1991b; Chen *et al.*, 1994). FECT has been used for quantitative measurements, and the Kato technique in large-scale surveys.

Qualitative diagnosis based on a single reading with Stoll's technique and FECT is highly sensitive (nearly 100%) in people with 20 worms or more, but the sensitivity of a single

reading drops to as low as 20% in people with fewer than five worms (Sithithaworn *et al.*, 1991b). Multiple reading of the same sample increases sensitivity up to 20% (Haswell-Elkins *et al.*, 1994a). The sensitivity of the diagnostic techniques used in epidemiological studies determines accurate assessment of prevalence and the effects of intervention.

In patients whose bile ducts are completely obstructed, eggs occur in the gall-bladder bile, and serological methods may be used to determine infection (Kurathong *et al.*, 1985; Pungpak *et al.*, 1985).

Eggs of minute intestinal flukes, e.g. species of *Heterophyes*, *Phaneropsolus* and *Haplorchis*, can be confused with those of *Opisthorchis* and *Clonorchis* (Ditrich *et al.*, 1990; Giboda *et al.*, 1991a; Kaewkes *et al.*, 1991; Ditrich *et al.*, 1992).

1.2.2 *Quantitative faecal assessment of intensity of infection*

The combination of egg counts with worm recovery after treatment is the optimal procedure for assessing intensity of infection (Haswell-Elkins *et al.*, 1994a), as there is a close relationship (Radomyos *et al.*, 1984; Sithithaworn *et al.*, 1991b). Studies of autopsy specimens show, however, that people with high egg counts sometimes do not expel eggs (Ramsay *et al.*, 1989; Elkins *et al.*, 1991).

Daily variation in faecal egg output appears to be minimal and does not greatly affect the accuracy of different techniques (Viyanant *et al.*, 1983; Kurathong *et al.*, 1984; Pungpak *et al.*, 1990).

1.2.3 *Serological tests for helminth-specific antibody and antigen*

Immunodiagnostic tests for liver fluke infections are considered to be supplementary tools rather than definitive diagnostic assays (Rim, 1986; Sirisinha, 1986). Their use in epidemiological surveys is limited, owing to lack of specificity, lack of differentiation of past and present infections and limited sensitivity (Viyanant *et al.*, 1985; Chen *et al.*, 1987; Hong, 1988; Thammapalerd *et al.*, 1988; Wongratanacheewin *et al.*, 1988).

Serological methods may be preferable in some circumstances, as they indicate exposure that occurred before antihelminthic treatment.

Comparisons between the enzyme-linked immunosorbent assay (ELISA), immunofluorescence, complement fixation and indirect haemagglutination for the detection of antibodies against *Opisthorchis* and *Clonorchis* show that ELISA is usually the most sensitive and specific. A sensitivity of 90.2% and a specificity of 84% was seen for *Clonorchis* in a comparison of infected people with people outside an endemic area (Chen *et al.*, 1988). Cross-reactions in crude ELISAs have been reported in sera from patients with intestinal nematodes, schistosomiasis, angiostrongyloidiasis, paragonimiasis and minute intestinal fluke infection (Chen *et al.*, 1988; Poopyruchpong *et al.*, 1990; Ditrich *et al.*, 1991).

Comparisons of antibody responses to crude somatic extracts among infected and uninfected individuals within endemic communities show significant associations between infection and the frequency and level of helminth-specific antibody (Janechaiwat *et al.*, 1980; Srivatanakul *et al.*, 1985; Poopyruchpong *et al.*, 1990; Haswell-Elkins *et al.*, 1991a). The sensitivity of antibody tests in light infections is limited (Haswell-Elkins *et al.*, 1991a), and intensity of infection cannot be inferred from antibody level (Rim, 1986; Haswell-Elkins *et al.*, 1991a).

Chen *et al.* (1987) described a sandwich ELISA for detecting circulating antigen in sera of people infected with *C. sinensis*. ELISAs that include a mixture of helminth-specific monoclonal antibodies can be used to detect *O. viverrini* antigens in faeces, while a 340-base-pair DNA probe can be used to detect helminthic DNA in faeces (Sirisinha *et al.*, 1991, 1992).

1.2.4 *Intradermal tests*

Skin testing with a diluted extract of adult *C. sinensis* antigens has been used widely in China and the Republic of Korea in epidemiological and surveillance studies, but this method is less commonly used today. The estimated specificity and sensitivity of the reaction was reported to be 98% by Komiya (1966), but lower values (83 and 78%) were reported subsequently (Rim, 1986).

1.3 Epidemiology of infection

1.3.1 *Geographical distribution*

The worldwide distribution of *O. viverrini*, *O. felineus* and *C. sinensis* is shown in Figures 2 and 3.

Countries in which human liver fluke infection is endemic are China, Japan, the Republic of Korea, Laos, Thailand, Viet Nam, the Russian Federation, the Ukraine and parts of eastern Europe. A very crude estimate of the global number of infections is of the order of 17 million, comprising seven million with *Clonorchis*, nine million with *O. viverrini* and 1.5 million with *O. felineus* (WHO, 1994). Regional and global migration of peoples has broadened the distribution of the helminths. Since their life cycles usually do not become established, this widened distribution has limited epidemiological relevance, but, given the potential severity of disease, it is of clinical importance (Chan & Lam, 1987). As details of the sampling methods and examination techniques used are sometimes omitted from survey reports, the sensitivity and representativeness of the measurements cannot be evaluated.

(*a*) *Opisthorchis viverrini*

The first studies of the epidemiology of *O. viverrini* in North-east Thailand, for which relatively insensitive diagnostic methods (simple smears and Kato technique) were used, suggested that about one-third of people in the region harboured infection (Sadun, 1955; Wykoff *et al.*, 1965; Harinasuta, 1969). A survey summarized by Harinasuta (1969), in which the FECT method was used, showed, however, that more than 60% of a sample drawn from 15 north-eastern provinces was infected. Higher prevalences (up to 92%) were seen in the northern provinces that border Laos, and lower prevalences (as low as 10%) in the southern provinces, which border Cambodia. More recent surveys have shown that *O. viverrini* still infects about 15% of the Thai population of 58 million, and about 24% of the North-east Thai population of 20 million (Jongsuksantigul *et al.*, 1992). The level of infection in northern Thailand is less clear, owing to its uneven distribution. Harinasuta (1969) reported prevalences of over 15% in three of 17 provinces (Chiang Mai, Prae and Lampang); Preuksaraj (1984) noted similar levels only in Sukhothai (22%) and Phetchabun (17%). The most recent survey in northern Thailand (Jongsuksantigul *et al.*, 1992) showed an overall average prevalence of 23% which, if substantiated, suggests an increase in prevalence.

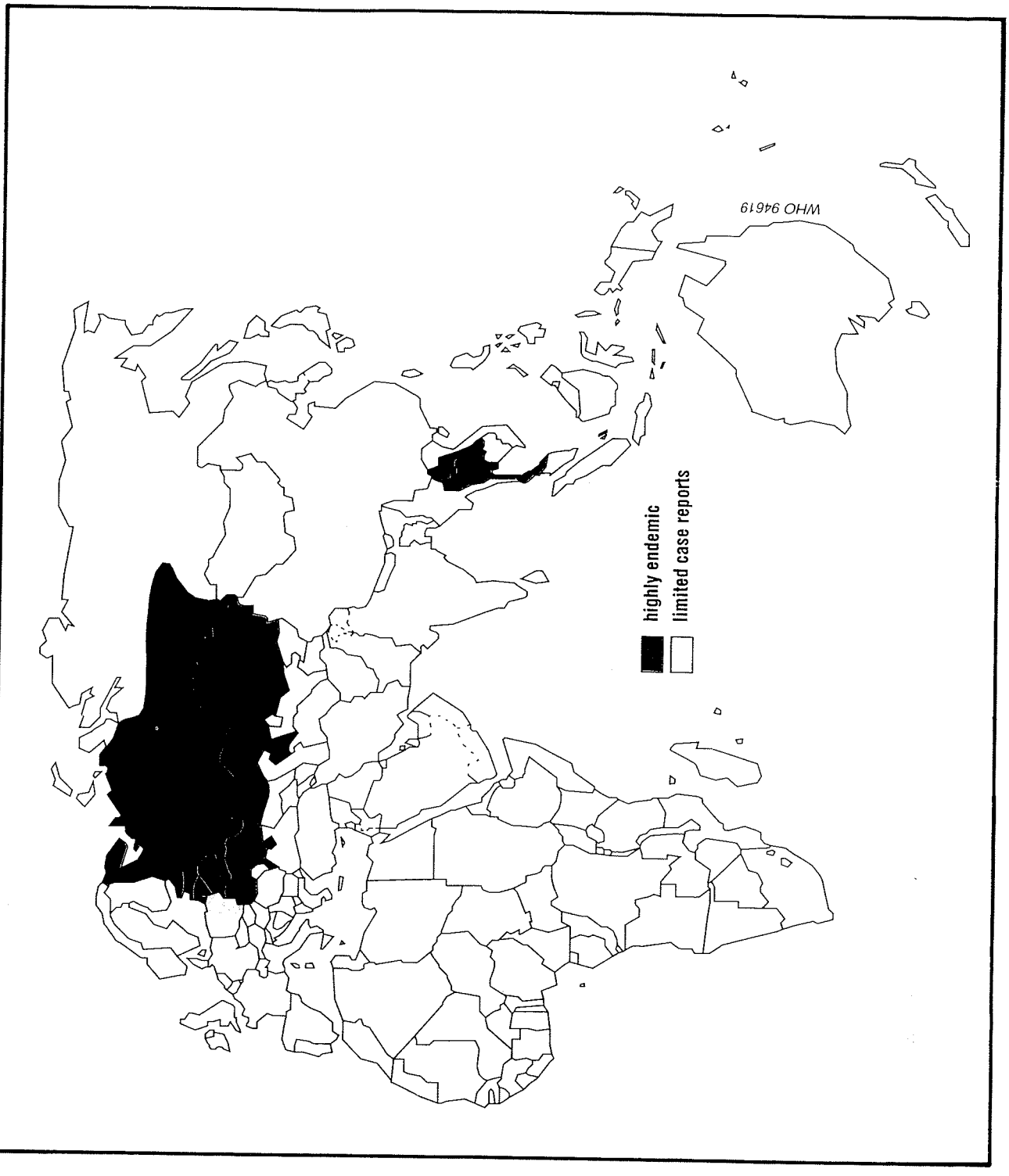
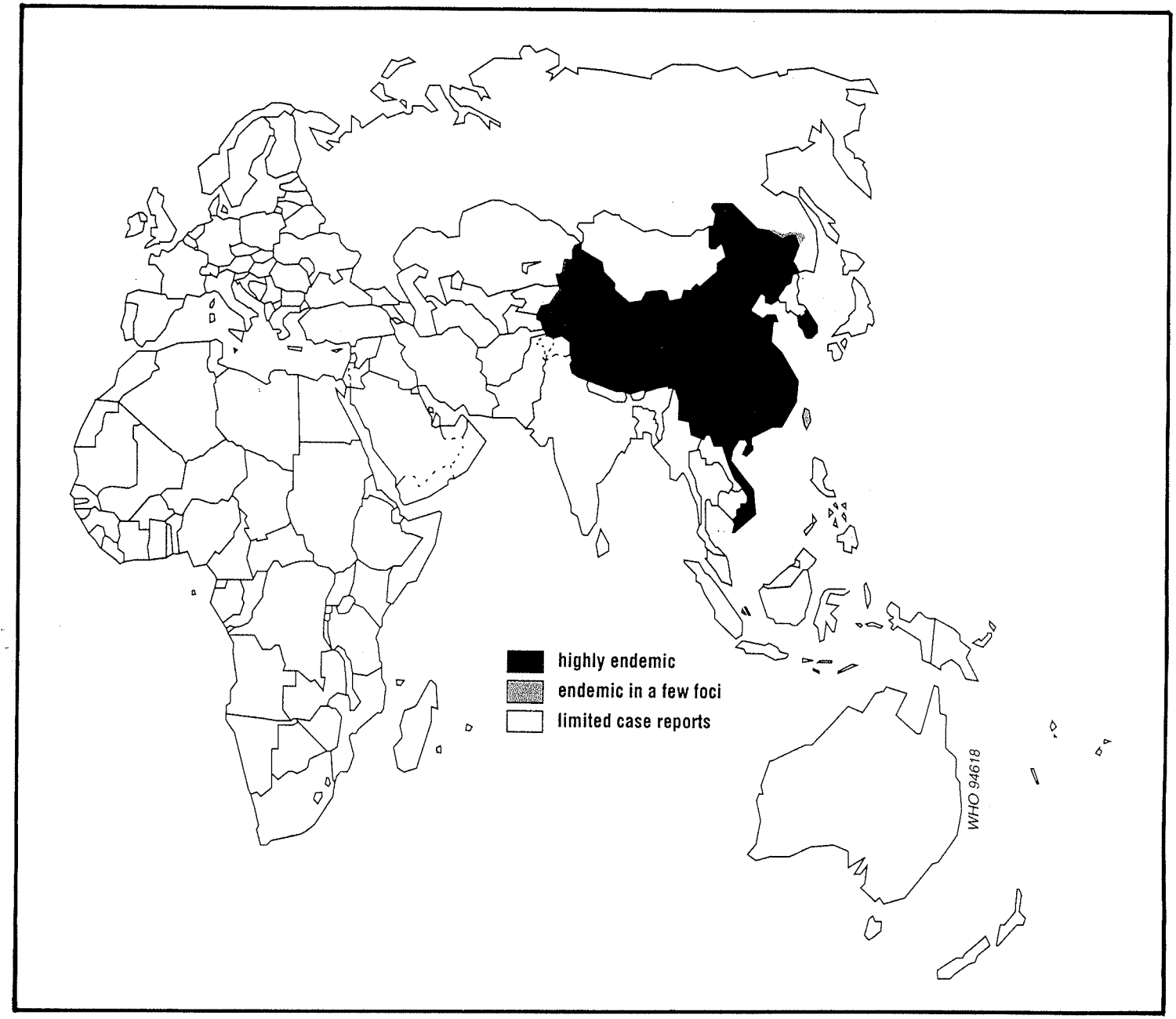


Figure 2. Worldwide distribution of *Opisthorchis viverrini* and *O. felineus*

Figure 3. Worldwide distribution of *Clonorchis sinensis*



The helminth is common in the lowlands of Laos among people with close ethnic ties to the majority of the North-east Thai population; however, the total number of infections is not known (Giboda *et al.*, 1991b; Pholsena *et al.*, 1991).

(*b*) *Opisthorchis felineus*

About 1.5 million cases of *O. felineus* infection are seen in the former USSR, according to a tabulation of the results of surveys prepared in 1992 for WHO (Iarotski & Be'er, 1993). Some 1.2 million infections are estimated to occur in the Russian Federation, as projected from a total of 78 400 that were officially registered. Infections are registered in 24 of the 73 territories in the Federation, mostly in western Siberia and particularly along the valleys of the Ob' and Irtys' rivers and their tributaries; the largest number of registered infections (and over 900 000 extrapolated cases) were reported from the two districts of T'umen' and Tomsk. High prevalences were also observed in areas along the Volga–Kama river basin, and along river basins in the the Novosibirsk, Krasnojarsk, Kurgan, Kemerovo, Sverdlovsk, Omsk and Tomsk districts and the Altaj territory (Klimshin *et al.*, 1981; Iarotski & Be'er, 1993).

Infections also occur in the Ukraine and Kazachstan. Reports from the Ukraine indicate that infection is found in the Sumy, Poltava and Černigov districts within the Dnepr River basin, with prevalences of 5–40%. In Kazachstan, the average prevalence in six endemic districts was less than 10% (Iarotski & Be'er, 1993).

Eight percent of the population of one rural area in Germany was reported to be infected in 1929, but more recent surveys on parasitic zoonosis in this region indicated that the infection no longer persists (Hinz, 1991).

(*c*) *Clonorchis sinensis*

C. sinensis is distributed in reservoir hosts throughout China, but human infection is largely confined to 24 provinces and municipalities in the south and north-east, as delineated by the eating of raw or undercooked fish. This behaviour and the infection are ethnically and geographically associated; the most frequent consumers and infections in the south occur among the Cantonese, particularly the Hakka people (Rim, 1986), and those in the north-east occur among the Korean national minority who migrated there (Chen *et al.*, 1994).

Clonorchis is commonest in Guangdong and Guanxi Zhuang provinces in the south, where four million people are thought to be infected (Li, 1991; Chen *et al.*, 1994; Fang, 1994). The highest infection levels in Guangdong Province are observed in the Pearl River delta (with an estimated prevalence of 21.1% based on surveys between 1973 and 1991), the upper reaches of the Pearl River (4.4%) and the Han River drainage basin (5.1% infected) (Fang, 1994). The You River runs through the areas of highest prevalence of infection in Guanxi, where some 7.3% of inhabitants are infected. Other endemic provinces in China include Heilongjiang, Jilin and Liaoning in the north-east, Jiangsu along the Yangtze River and periurban areas of Beijing where fish are abundant in canals (Chen *et al.*, 1994).

Infection in Hong Kong is probably acquired by eating fish imported live from Guangdong Province in southern China, since no infection has been found among local snails. Estimated prevalences of infection in Hong Kong, with its large Cantonese population, range from as high as 46–65.6% (Hou & Pang, 1964; Belamaric, 1973) to 23% (Attwood & Chou, 1978). Eggs were found in 13.4% of simple faecal smears (an insensitive

method) of Hong Kong residents applying to emigrate to Canada (Ko, 1991). The populations sampled in these surveys, however, are not random, so that the true prevalence may be overestimated. For example, since imported fish are expensive, the prevalence may be higher among wealthier residents (Chen *et al.*, 1994) who might be more likely to apply to go abroad.

Clonorchis infection is distributed throughout Taiwan, at prevalences ranging from < 1 to 57%. Heavy infection is frequent among Hakka people who emigrated from Guangdong Province to the Mei-Nung and Kaohsiung districts in southern Taiwan (Komiya, 1966; Hou *et al.*, 1989; Chen, 1991). The Miaoli district in the north and the Sun-Moon Lake area in the central part are also important endemic areas, where 20–50% of the population are infected (Chen, 1991). The endemic area may be increasing as new areas are reporting significant prevalences of infection.

Infections have largely been eliminated in Japan, where highly endemic areas were reported in the 1960s in several river basins (Chen *et al.*, 1994). The prevalence and intensity have since dropped steadily, and *Clonorchis* may now be almost eradicated (Rim, 1986), largely due to improvements in sanitation and health education.

Infection in the Republic of Korea has been documented extensively. In the past, both prevalence and intensity were high: in a nationwide survey in 1959, up to 15% of the population responded positively to skin testing (Chen *et al.*, 1994). The highly endemic areas occurred in seven river basins, in which community prevalences were 30–80% (Elkins *et al.*, 1994). Large-scale control activities under way since 1984 have decreased the prevalence to 2.2% (Ministry of Health and Social Affairs, 1992).

High prevalences of infection were also reported in the past in northern Viet Nam, in the Red River delta near Hai Phong and Ha Noi; however, *Clonorchis* infection was rare in the south (Rim, 1986). A survey among 968 inhabitants of Ha Nam Nin province showed a prevalence of 28.4% (Lam *et al.*, 1990).

Clonorchis has also been reported in the Amur River basin in the far eastern region of the Russian Federation, where it infects some 24% of the aboriginal population (the Nanaians) (Sergiev *et al.*, undated).

The prevalence of infection with *Opisthorchis* and *Clonorchis* in places like Hong Kong and Macao, where most freshwater fish is imported, depends on the origin of the fish.

1.3.2 *Risk factors for infection*

(*a*) *Opisthorchis viverrini*

In North-east Thailand, three types of preparations contain uncooked, usually small fish: fresh (*koi-pla*; eaten the same day), moderately fermented (*pla-som*, salted and stored for five days to three months) or completely fermented (*pla-ra*, highly salted, stored for two to three months to over one year) (Sadun, 1955). In the past, reported consumption frequencies of *koi-pla* were very high: up to 80% in some communities ate the dish on a weekly basis (Migasena, 1982). In a comparison of rural and urban dwellers, Kurathong *et al.* (1987) reported higher prevalences of liver fluke infection among rural than urban residents from the north-east region and among rural residents who reported having eaten *koi-pla* (87%) than among those who did not (61%). Upatham *et al.* (1984) reported a closer relationship

with *koi-pla* consumption within a heavily infected village, with only 19% of uninfected people, 79% of infected people and > 90% of heavily infected people reporting consumption.

More recent surveys suggest that the frequency of *koi-pla* consumption has declined and is generally confined to special social occasions, while uncooked *pla-som* is generally eaten several times a week (Changbumrung *et al.*, 1989). Fully preserved fish (e.g. *pla-ra*) is an important staple food, consumed daily by 80–99% of north-eastern Thais descended from Laotians (Migasena, 1982; Changbumrung *et al.*, 1989). It is commonly believed that liver fluke infection can occur from eating any of these dishes, but the infectivity of the various preparations remains unclear. Several studies have indicated that survival of the infective stages depends on the concentration of salt and the degree of fermentation (Tesana *et al.*, 1986). These findings suggest that *koi-pla* is probably the most infective, followed by fish preserved for less than seven days, while viable metacercaria would be very rare in *pla-ra*.

(*b*) *Opisthorchis felineus*

Fish is a major source of food in western Siberia and other endemic areas of the former USSR, where people eat uncooked fish, frozen, salted and smoked; frozen fish is sliced and eaten with condiments. Aboriginal inhabitants (Ugro-Finn, Khanti, Mancy, Nencie) eat raw fish, as do 10–40% of migrants into the endemic areas, e.g. miners, geologists and labourers, who become infected with *O. felineus* within one to two years (Iarotski & Be'er, 1993).

(*c*) *Clonorchis sinensis*

In southern China and among the Cantonese of Hong Kong, raw fish is traditionally eaten after being dipped in rice porridge (Chen *et al.*, 1994). Alternatively, large fish are sliced and eaten with ginger and garlic. Higher levels of infection and poorer nutritional status were reported among children in hilly areas of Guangdong Province than among those living along rivers, while infection patterns among adults show the opposite trend. This observation led to the finding that children in the hilly areas often catch fish during play and roast them incompletely before eating. As they grow older, they catch fish less frequently than adults living on riversides, and the intensity of infection declines. Koreans eat raw fish soaked in vinegar, red-pepper mash or hot bean paste with rice wine at social gatherings (Choi, 1984). The fact that men do so more frequently than women has been given as an explanation for higher prevalences of infection among men; however, in heavily infected areas, there is often no difference between the sexes. When fish are abundant, raw fish is eaten commonly rather than being reserved for special occasions (Rim, 1986). Vietnamese people eat raw fish in salads (Kieu *et al.*, 1990).

Infection in Japan, which is now very rare, appeared to come from frequent consumption of slices of large, raw, freshwater fish with vinegar or soya bean paste (Chen *et al.*, 1994). In contrast, smaller co-existing species were rarely eaten uncooked. The large fish, namely *Cyprinus carpio* and *Carassius carassius*, were infrequently and lightly infected with metacercariae, possibly because of the presence of toxic components in their mucus (Rhee *et al.*, 1988). *Sushi* and other preparations of uncooked seafish eaten in Japan today do not carry *Clonorchis*.

1.3.3 *Age- and sex-related patterns of infection*

While the levels of *O. viverrini* infection vary considerably between villages in Thailand, the patterns of infection are fairly similar. In general, the youngest age groups (often 0–4 years) show low prevalence and intensity, while these increase in the pre- and early teens and often reach a plateau in late teenage groups (i.e. 15–19). In some areas, the intensity of egg excretion continues to increase with age (Upatham *et al.*, 1982), while the worm burden may decline (Haswell-Elkins *et al.*, 1991b; Sithithaworn *et al.*, 1991a).

Anecdotal descriptions have been reported of mothers in the Republic of Korea and Thailand feeding raw fish to their infants (Choi, 1984), and infections have been observed in young infants (Sadun, 1955; Harinasuta & Vajrasthira, 1960; Upatham *et al.*, 1982, 1984). The reported intensities of infection in children under the age of four are, however, invariably very low, and there is little evidence that young children have ever had frequent, intense exposure to infection.

The prevalence and average intensity of *O. viverrini* infection do not usually differ, or are slightly higher, among males than females (Wykoff *et al.*, 1966; Upatham *et al.*, 1982, 1984; Haswell-Elkins *et al.*, 1991b; Elkins *et al.*, 1994). Even in areas where these measurements do not differ significantly with sex, higher frequencies of heavy infection may be observed among males (Haswell-Elkins *et al.*, 1991b; Elkins *et al.*, 1994).

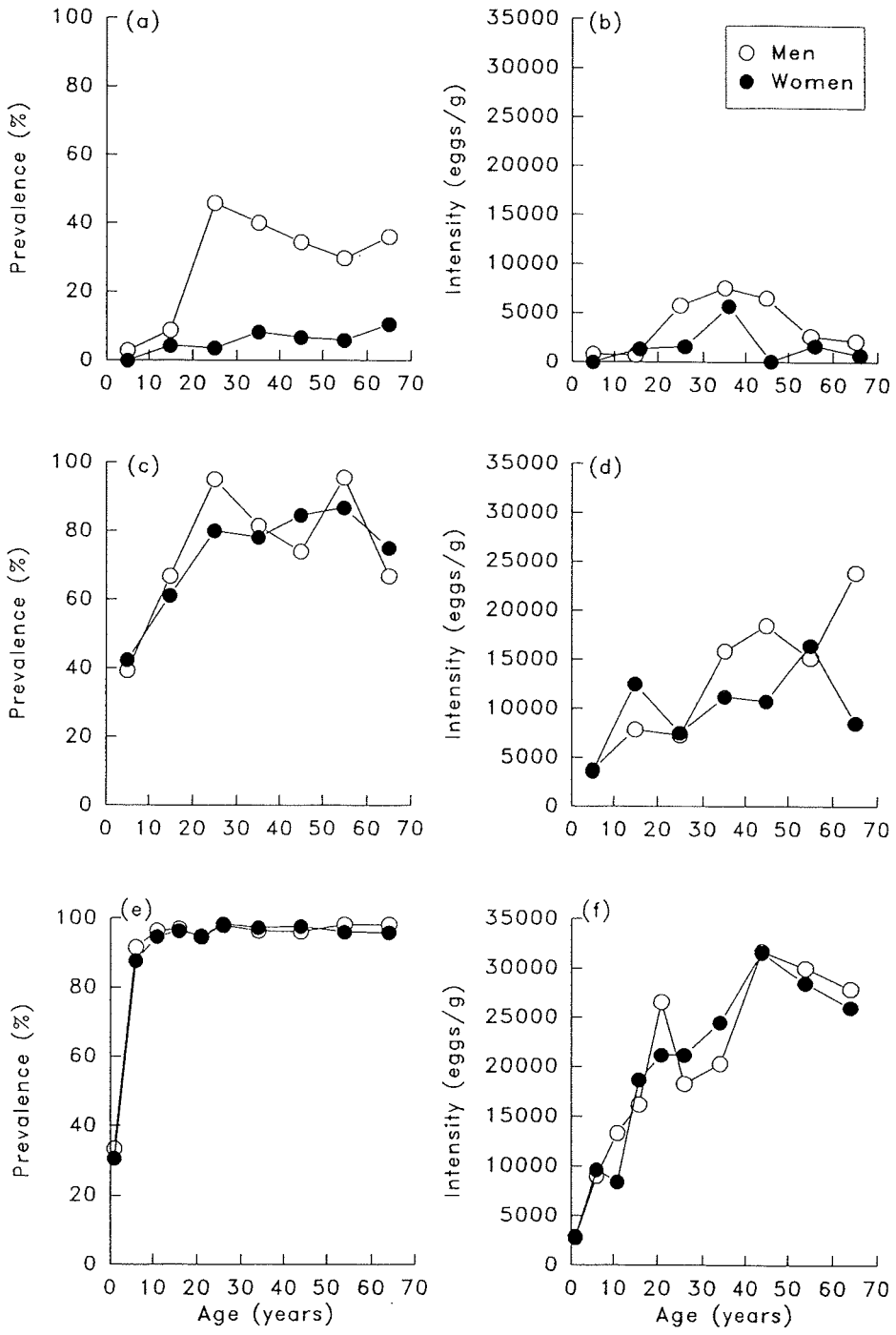
In general, the prevalence of *Clonorchis* infection appears to rise at later ages, and differences in prevalence and intensity between the sexes are more pronounced than those of *Opisthorchis* (Figure 4). For example, in several river basins in the Republic of Korea, large increases in prevalence are observed between the ages of 10–19 and 20–29. Sometimes this is apparent only in males, and females maintain relatively low prevalences throughout life, while in other areas the two sexes have virtually identical age-related patterns of infection. Most studies in Japan show maximal prevalences at 30–50 years of age (e.g. 39–67% and 8.8–46%) (Rim, 1986). This finding appears to be generally true in China, except in areas where children become infected by catching and eating undercooked fish during play (Chen *et al.*, 1994).

1.3.4 *Aggregation of infection*

The population of *O. viverrini*, and probably all three liver flukes, is highly aggregated within a small minority of people who are heavily infected. For example, Haswell-Elkins *et al.* (1991b) observed that 81% of 11 027 worms recovered after treatment of 246 village residents were expelled by just 27 individuals with burdens of over 100 worn Similarly, Sithithaworn *et al.* (1991a) reported that 30 of 181 cadavers examined contained 66% of all worms recovered at autopsy.

The levels of infection vary considerably between communities within the same province and district, for unknown reasons. Tesana *et al.* (1991) found higher prevalences of infection in six villages located far from a river than in villages situated along river banks. This observation is in contrast to the patterns usually reported for *Clonorchis* infection and may reflect variation in the habitats of infected fish.

Figure 4. Prevalence (a) and intensity (b) of infection with *Clonorchis sinensis* in an area of low intensity in the Republic of Korea; prevalence (c) and intensity (d) of infection with *C. sinensis* in an area of high intensity in the Republic of Korea; prevalence (e) and intensity (f) of infection with *Opisthorchis viverrini* in an area of high intensity in Thailand



Intensities are arithmetic means. (a)-(d) from Rim (1986); (e)-(f) from Upatham *et al.* (1994)

1.4 Clinical disease in humans (other than cancer)

The frequency and types of clinical disease appear to differ for the three human liver flukes. Most notably, reports in the Russian literature give specific signs and symptoms for well-defined clinical stages of opisthorchiasis, from acute to chronic (Bronshtein, 1986). Acute infection, characterized by high fever, hepatitis-like symptoms and eosinophilia, is frequently reported in *O. felineus* infections but has been documented infrequently for clonorchiasis (Rim, 1986) and for *O. viverrini* infections. This finding may be due to the fact that a large number of migrants enter the area endemic for *O. felineus* and become infected as adults; this pattern is unusual in infections with the other two liver flukes.

Much of the published information comes from uncontrolled clinical investigations, e.g. case studies and reviews of hospital records, which do not include a control group for comparison (Markell, 1966). Furthermore, since most of the studies have been hospital-based, the frequencies with which these clinical manifestations occur during the course of infection cannot be inferred. As a result, there has been a strong tendency to overestimate both the frequencies and strengths of association between the infections and various presentations (Markell, 1966; Woolf *et al.*, 1984).

Two large studies (Upatham *et al.*, 1982, 1984) within a heavily infected community reported significantly increased frequencies of abdominal pain in the right upper quadrant, flatulence or dyspepsia and weakness associated with increasing intensity of infection. They estimated that 5–10% of the community had symptoms attributable to the infection.

Most other clinical and laboratory assessments show little or no difference in liver function, nutritional status or clinical signs and symptoms between infected and uninfected individuals, and no difference following anthelminthic treatment (Pungpak *et al.*, 1990). Total serum IgE, white blood cell count and percentage of eosinophils are often elevated, but this finding may sometimes be confounded by other infections (Joo & Rim, 1982).

Increased levels of serum protease inhibitors (α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin) (Changbumrung *et al.*, 1982), of three serum bile acids (taurocholic acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid) (Migasena *et al.*, 1983) and of the activities of a number of hepatic enzymes (Pongpaew *et al.*, 1985) have been reported among people with *O. viverrini* infection. Migasena *et al.* (1983) reported an increase in the trihydroxy:dihydroxy ratio and in total bile acids with intensity of egg output, which is a stronger indication of association with infection.

Schelp *et al.* (1974) similarly observed no difference in nutritional, clinical or haematological status between infected and uninfected individuals in a village in North-east Thailand. They did, however, find an increase in the ceruloplasmin and haemopexin peak and in haptoglobin levels among infected people, which they suggested was due to bile retention in liver cells and inflammation. Analyses were not done after treatment.

Studies using ultrasonography have shown strong relationships between intensity of infection and gall-bladder enlargement, gall-bladder wall irregularities and sludge, and enhanced echogenicity of the portal triad (Dhiensiri *et al.*, 1984; Mairiang *et al.*, 1992). These abnormalities were reversible within 10 months after praziquantel treatment (Mairiang *et al.*, 1993).

The presence of stones in the gall-bladder, liver and bile ducts has frequently been linked to *Clonorchis* infection; the best evidence is the finding of eggs or worm fragments in the nidus (Teoh, 1963). Hou *et al.* (1989) reported a consistent increase in gallstone frequency (diagnosed by ultrasound) with increasing intensity of infection among Hakkanese people in Taiwan, from 4.2% in uninfected subjects to over 14% in those who excreted more than 5000 eggs/g. Similar clinical findings have been reported infrequently in cases of *Opisthorchis* infection (Riganti *et al.*, 1988).

Ascending cholangitis and obstructive jaundice are common complications of opisthorchiasis. Pungpak *et al.* (1985), however, reported only 88 cases of severe manifestations among 15 243 infected people who attended a hospital in Bangkok. These manifestions included obstructive jaundice and cholangitis; at least 16 patients had cholangiocarcinoma. Since radiological investigations were not performed, cholangiocarcinoma could not be ruled out as a cause of the manifestations.

1.5 Treatment and control

Clonorchis has been successfully controlled in Japan, and the current prevalence in the Republic of Korea is 2.2% (Ministry of Health and Social Affairs, 1992). In other areas, it is often difficult to assess the success of control efforts, owing to lack of epidemiological data. The main tools that have been used in control programmes have been anthelminthic treatment, improved sanitation and health education. The rationale is that treatment is required to eliminate the long-lived parasites immediately, sanitation interrupts transmission from human faeces to snails, and health education stops people from eating raw fish and becoming reinfected after treatment. A number of studies have suggested that control programmes involving treatment and health education are more effective in suppressing reinfection than treatment alone (Sornmani *et al.*, 1984; Saowakontha *et al.*, 1993). Community participation in the planning and implementation of control programmes is a vital element in their success (Keittivuti *et al.*, 1986; Sornmani, 1987).

Strategies that have been suggested but not widely implemented include destroying metacercaria in fish through irradiation (Lee *et al.*, 1989; Sornmani *et al.*, 1993) and deep-freezing (Song, 1987; Iarotski & Be'er, 1993), applying molluscicides, using biological agents (*Mesocyclops leuckari*) to destroy cercariae (Intapan *et al.*, 1992) and treating reservoir hosts. Improvements in sanitation, by supplying latrines and stopping the use of night-soil as fertilizer on fields and as food for fish, have been widely implemented. No progress has been reported towards development of a vaccine.

Control efforts are influenced by the massive environmental changes that are occurring in many endemic areas, notably China, Japan, the Repubic of Korea and Thailand. As natural aquatic life is affected by pollution, fish become less abundant and the life-cycle is disrupted (Choi, 1984; Joo, 1988).

The single dose of praziquantel generally used for *O. viverrini* and *C. sinensis* infections in the Republis of Korea and Thailand is 40 mg/kg bw, while higher, multiple doses (3 × 25 mg/kg bw for one to three days) have been used for treatment in China. Although the drug has a number of side-effects, these are transient and relatively minor. The published efficacy of this dosage is invariably over 90% (Vivatanasesth *et al.*, 1982; Chen *et al.*, 1983; Rim, 1986;

Viravan *et al.*, 1986). Reinfection can occur after treatment. Upatham *et al.* (1988) reported an extremely rapid return (less than one year) to pre-treatment levels of infection in a mass-treated community that had had an extremely high initial intensity of infection. Furthermore, these authors showed a significant association between pre- and post-treatment egg counts among individuals, indicating that stable, individual behavioural and immunological factors, as well as chance, determine levels of infection.

2. Studies of Cancer in Humans

2.1 Descriptive studies

The association between liver fluke infection and the occurrence of cancer in humans has been reviewed extensively (Stewart, 1931; Higginson, 1955; Yamagata & Yaegashi, 1964; Gibson, 1971; Tansurat, 1971; Viranuvatti & Stitnimankarn, 1972; Schwartz, 1980; Flavell, 1981; Juttijudata *et al.*, 1984; Kim, 1984; Chan & Lam, 1987; Haswell-Elkins *et al.*, 1992a,b; Parkin *et al.*, 1993; Sithithaworn *et al.*, 1994).

2.1.1 *Opisthorchis viverrini*

All of the available studies are from Thailand, where there is substantial geographical variation in the prevalence of infection, increasing from the south to the north, the highest rates being observed in Khon Kaen Province in North-east Thailand (see section 1.3.1a). In incidence data from the national cancer registry, the highest frequency was observed in North-east Thailand in 1980–82 (Srivatanakul *et al.*, 1988) and again, especially in Khon Kaen Province, in 1988–91 (Vatanasapt *et al.*, 1993). In the earlier period, the proportionate incidence ratio was 3.1 (95% confidence interval [CI], 2.8–3.5) for cholangiocarcinoma and was 1.2 (95% CI, 1.1–1.4) for hepatocellular carcinoma (Srivatanakul *et al.*, 1988). In Khon Kaen Province around 1985, the age-standardized incidence rate of cholangiocarcinoma was 84.6 per 100 000 per year in men and 36.8 per 100 000 per year in women. Outside of Thailand, the incidence of cholangiocarcinoma shows little variation (range, 0.2–2.8 per 100 000 per year in men, and 0.1–4.8 per 100 000 per year in women) (Parkin *et al.*, 1993). Thus, the incidence in the area of highest incidence in Thailand is at least 40 times as high as that in the area of highest incidence elsewhere.

Within Khon Kaen Province, during the period 1985–88, Vatanasapt *et al.* (1990) observed the highest incidence and mortality rates of liver cancer in three adjacent districts; studies in two of the districts had shown high prevalences of infection and heavy infection (Upatham *et al.*, 1984). Subsequently, Sriamporn *et al.* (1993) showed that there was no difference in the overall prevalence of infection between the districts of highest and lowest incidence of liver cancer within the Province during the period 1988–90; however, 9% of 331 subjects from randomly selected villages in the district of highest incidence had > 10 000 fluke eggs/g of stool, while only 3.7% of 296 subjects in villages in the district of low incidence had the same level of infection.

Srivatanakul *et al.* (1991a) carried out a correlation analysis of liver cancer incidence, titre of antobodies to *O. viverrini* and faecal egg count (determined in healthy volunteers who

had been born and resided in the area) in five regions with different frequencies of the two main histological types of liver cancer: Chiang Mai in the north, Nakon Ratchasima and Ubon Ratchathani in the north-east (but not in Khon Kaen Province), Bangkok in the centre and Songkhla in the south. The correlation between the incidence of cholangiocarcinoma and the proportion of subjects with an antibody titre $\geq$ 1:40 was 0.98 ($p = 0.004$), and that with faecal egg count was 0.53 ($p = 0.35$). For hepatocellular carcinoma, which showed little geographical variation in incidence, the correlations were –0.37 ($p = 0.54$) and 0.02 ($p = 0.96$), respectively. [The weaker association between cholangiocarcinoma and faecal egg count may reflect the introduction of effective therapy; antibody titre is thought to provide a more valid indicator of past infection, but cross-reactivity with other parasites common in the region may have been involved.]

These studies are summarized in Table 1.

2.1.2 *Opisthorchis felineus*

In the T'umen' region in western Siberia (an area of *O. felineus* endemicity), Shain (1971) related the prevalence of infected people in four subregions as reported by local health centres with the incidence of liver cancer observed in the same period, 1960–69. The correlation computed by the Working Group from the tabulated data was [0.98; $p < 0.05$]. A similar analysis in seven cities within one of the regions confirmed this correlation [0.77]. No information was given on the relative frequency of histological types.

2.2 Case reports and case series

2.2.1 *Opisthorchis viverrini*

All of the available reports are from Thailand. The earliest case reports are of a papillary adenocarcinoma of the liver and an adenocarcinoma of the bile duct (Viranuvatti & Mettiyawongse, 1953) and a retention cyst of the liver caused by opisthorchiasis associated with carcinoma of the liver (Viranuvatti *et al.*, 1955); *O. viverrini* infection was detected at autopsy in each case. Subsequent case series are summarized in Table 2. Among patients from the area in which *O. viverrini* is endemic, cases of cholangiocarcinoma outnumber cases of hepatocellular carcinoma, in contrast to other series.

Cancers other than of the liver have been reported in association with this infection, but no particular type has predominated (Koompirochana *et al.*, 1978; Pungpak *et al.*, 1985).

2.2.2 *Opisthorchis felineus*

Three studies on the presence of *O. felineus* infection in liver cancer cases were conducted in western Siberia (Table 3). One of the regions, T'umen', is reported to be an area of high endemicity. The prevalence of infection in 250 histologically verified cases of liver cancer was 52% in the study of Shain *et al.* (1971). The prevalence of infection in 44 cases of liver cancer detected in 657 autopsies performed in the same region was 95% (Glumov *et al.*, 1974). The first study also reported a higher frequency of cholangiocarcinoma among infected liver cancer cases and a difference in the sex ratios between the two main histological types [no information was provided about the sex ratio of infection].

Table 1. Descriptive studies of *Opisthorchis viverrini* and liver cancer in Thailand

Reference	Area and period of study	Details of cases of liver cancer			Measure of exposure to *O. viverrini*	Number of geographical units	Association	Comments
		Deaths or incidence	Type	Number				
Srivatanakul *et al.* (1988)	Whole country, 1980–82	Incidence	Liver cancer CCA HCC	3820 523 779	–	10 9 9	Highest PIR for liver cancer (men, 2.0; 95% CI, 1.9–2.2; women, 2.7; 95% CI, 2.4–3.0) observed in Khon Kaen Province in North-east Thailand. Highest PIR (3.1, 95% CI, 2.8–3.5) for CCA observed in North-east Thailand. Corresponding PIR for HCC was 1.2 (95% CI, 1.1–1.4).	
Vatanasapt *et al.* (1993)	Four population-based cancer incidence registries, 1988–91	Incidence	Liver cancer	4314	–	4	Highest incidence for CCA in Khon Kaen Province in North-east Thailand	
Vatanasapt *et al.* (1990)	Khon Kaen Province, 1985–88	Incidence Deaths	Liver cancer Liver cancer	1338 NR	–	20	Highest incidence and mortality rates in three adjacent districts (Chonnabot, Nong Rua and Muncha Khiri), in which other studies showed high prevalences of infection and heavy infection	Rate of total cancers in these areas very high
Sriamporn *et al.* (1993)	Districts with highest (Chonnabot) and lowest (Ban Phang) incidence of liver cancer in Khon Kaen Province, 1988–90	Incidence	Liver cancer	140	Eggs/gram in stool samples from 627 subjects aged ≥30 from randomly selected villages in each district	2	No difference in overall prevalence of infection; 9% of subjects from district in high-incidence area had > 10 000 eggs/g, compared with 3.7% in the other district	No significant difference in age and sex distribution of subjects
Srivatanakul *et al.* (1991a)	Five areas with different frequencies of CCA and HCC, 1980–82, 1983–87, 1988, depending on area	Incidence	CCA HCC		Antibody titre and faecal egg count in about 100 volunteers aged 30–40 in each area	5	Positive correlation between proportion of subjects with antibody titre ≥1:40 and CCA (r = 0.98, p = 0.004). Correlation between eggs/g and CCA was 0.53 (p = 0.35). Corresponding correlations with HCC –0.37 (p = 0.54) and 0.02 (p = 0.96)	No strong or significant correlations between CCA and HBV infection, prevalence of HBsAg carriers, and aflatoxin levels in serum or urine

CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; PIR, proportionate incidence ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; NR, not reported

Table 2. Case series of patients with liver cancer associated with *Opisthorchis viverrini* infection in Thailand

Reference	Patients specified as coming from endemic area	Period of study	Cases				
			Method of ascertainment	Type	Number	*O. viverrini* infection	
						No.	%
Bhamrapravati & Virranuvatti (1966)	No	1960–62	Liver biopsy	HCC	251	5	2
				CCA	61	11	18
		1959–61	Autopsy	HCC	33	0	0
				CCA	14	11	79
Chainuvati *et al.* (1976)	Yes	NR	NR	Adenocarcinoma of cystic duct	4	3[a]	75
Koompirochana *et al.* (1978)	No	1954–74	Autopsy	HCC	266[b]	9	3.4
				CCA	108[b]	67	62
Sonakul *et al.* (1978)	No	17 years	Autopsy	HCC	9	From case series with *O. viverrini*	
				CCA	67		
	Yes	3 years	Autopsy	HCC	3	3	100
				CCA	8	8	100
Stitnimankarn *et al.* (1978)	Yes	NR	Liver biopsy	CCA	11	11	100
Pungpak *et al.* (1985)	No	1982–84	Autopsy, liver biopsy, surgery, ascitic fluids	Adenocarcinoma of liver	16	From case series with severe *O. viverrini*	
Riganti *et al.* (1989)	Yes	1969–88	Autopsy	Adenocarcinoma of bile duct	8	From case series with *O. viverrini*	
				HCC	2		

NR, not reported; HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma

[a]By stool examination; all were found to have infection when the ducts were examined histologically.

[b]Combining cases reported to have *O. viverrini* infection and those reported to be without the fluke

Table 3. Prevalence of *Opisthorchis felineus* in case series of liver cancer in western Siberia in the Russian Federation

Reference	Region	Endemicity	Cases				Results
			Method of ascertainment	Total no.	*O. felineus* infection		
					No.	%	
Shain *et al.* (1971)	T'umen'	High	Clinical	250	130	52	Sex ratio (M/F) in uninfected same as expected from literature, i.e. 2–6; sex ratio in infected was reversed [figures not given]. Cancers in uninfected patients mainly HCC; those in infected patients CCA: 4–5 times more frequent than HCC
Glumov *et al.* (1974)	T'umen'	High	Autopsy	44	42	95	35/44 CCA, frequency in infected not given. Prevalence of liver cancer at autopsy 6.7%; 0.7% in another pathology department
Iablokov *et al.* (1980)	Tomsk	Intermediate	Autopsy	103	7	7	In the whole series, 54% HCC and 46% CCA. Four infected cases had CCA; 3 had HCC.

HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma

In a similar study conducted in a region of intermediate endemicity, 7 liver cancers out of 103 detected at autopsy were infected with *O. felineus* (Iablokov *et al.*, 1980). Similar proportions of cases of cholangiocarcinoma (4/47) and hepatocellular carcinoma (3/56) were infected.

2.2.3 *Clonorchis sinensis*

The earliest case reports of primary liver cancer concerned Chinese subjects (Watson-Wemyss, 1919; Bentham, 1920; Nauck & Liang, 1928; Ch'in *et al.*, 1955). Subsequent case series, from Hong Kong and the Republic of Korea and among Asian subjects in the USA, are summarized in Table 4. Cases have also been described in immigrants to North America from China (Schwartz, 1986; Colquhoun & Visvanathan, 1987) and Laos (Drinka & Sheehy, 1985; Sher *et al.*, 1989; Ona & Dytoc, 1991). The only other population in which cases have been reported is that of Japan (Nakashima *et al.*, 1977).

2.3 Case–control studies

2.3.1 *Opisthorchis viverrini*

Kurathong *et al.* (1985) assessed the prevalence of cholangiocarcinoma and hepatocellular carcinoma during 1981–83 in 551 (47%) patients from the north-east (49.8% of those attending a hospital in Bangkok) who agreed to provide stool specimens, on the basis of which they were characterized for the presence of *O. viverrini* eggs. All 551 were screened for hepatobiliary tract diseases. Nineteen of 25 cases of cholangiocarcinoma and 9 of 12 of hepatocellular carcinoma had ova in the stools. The cases were diagnosed by a variety of methods, including ultrasound biopsy and hepatic angiography. The crude prevalence odds ratios were [1.3 (0.5–3.6)] for cholangiocarcinoma and [1.3 (0.3–4.7)] for hepatocellular carcinoma. [Use of controls with other hepatobiliary disease may have biased the results.]

A hospital-based case–control study of cholangiocarcinoma (Parkin *et al.*, 1991) and hepatocellular carcinoma (Srivatanakul *et al.*, 1991b) was carried out in Thailand, in which 103 cholangiocarcinoma patients and 65 hepatocellular carcinoma patients living in and originating from North-east Thailand were recruited in 1987–88 from among patients whose disease was diagnosed sequentially in three hospitals. One control was matched to each case for sex, age (within five years), residence and hospital of recruitment. Controls were selected from among patients affected by a variety of non-malignant diseases, considered not to be related to the consumption of alcohol or tobacco. Infection with *O. viverrini* was assessed in terms of an increase in titre of antibodies to *O. viverrini* in serum as observed by ELISA (Srivatanakul *et al.*, 1985). For cholangiocarcinoma, the matched estimate of the odds ratio obtained from the final multivariate model, including adjustment for consumption of 'sticky' rice and betel-nut chewing, was 5.0 (95% CI, 2.3–11.0). No association was seen with chronic carriage of hepatitis B virus nor with recent aflatoxin intake (Parkin *et al.*, 1991). *O. viverrini* infection was not significantly associated with the risk of developing a hepatocellular carcinoma. The observed odds ratio was 1.7 (0.8–3.7). In a multivariate analysis, there was a strong association with chronic carriage of hepatitis B virus (Srivatanakul *et al.*, 1991b).

Haswell-Elkins *et al.* (1994a) conducted a cross-sectional population-based survey in 1990–91 of subjects aged 25 or more from 46 villages in two districts of Khon Kaen Province

Table 4. Case series of patients with cancer of the liver associated with Chlonorchis sinensis infection

Reference	Location	Period of study	Cases				
			Method of ascertainment	Type	No.	*C. sinensis* infection	
						No.	%
Hou (1956)	Hong Kong	7 years	Autopsy	Adenocarcinoma (21) and mixed type of intrahepatic second-order bile duct tumours	30	30[a]	100
Belamaric (1973)	Hong Kong	1961–66	Autopsy	Adenocarcinoma of intrahepatic bile duct	19	18	95
Chou & Chan (1976)	Hong Kong	1964–73	Autopsy	CCA	50	46	92
Purtilo (1976)	Hong Kong	NR	Autopsy	CCA	7	From series of subjects with *C. sinensis* infection	
				HCC	10		
Ho (1980)	Hong Kong	Before 1976[b]	Autopsy	Mucoepidermoid carcinoma of the liver	2	0	0
Koo *et al.* (1982)	Hong Kong	1976–80	Laparotomy	Mucoepidermoid carcinoma of the bile duct	3	3	100
Kim *et al.* (1974)	Republic of Korea	1962–72	Autopsy				
	Seoul			HCC	339	28	8.3
				CCA	33	8	24.2
	Pusan			HCC	84	15	17.9
				CCA	21	13	61.9
Choi *et al.* (1988)	Republic of Korea	7 years	Surgery	CCA	16	10	62.5
Choi *et al.* (1989)	Republic of Korea	4 years	Surgery	CCA	20	From series of subjects with *C. sinensis* infection	
				HCC	4		
Strauss (1962)	USA, Asian subjects	1945–60	Surgery	Hepatomas	5	From series of subjects with *C. sinensis* infection	

NR, not reported; CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma

[a]Clonorchiasis; 28 (93%) cases were found to have flukes in the bile duct.

[b]Koo *et al.* (1982)

and 39 villages in Maha Sarakham Province, within the endemic area of *O. viverrini* infection in North-east Thailand. Stool specimens were obtained from 7727 subjects (participation rate, 72%) in Khon Kaen Province and 4585 subjects (participation rate, 79%) in Maha Sarakham Province after a health education programme about liver fluke infection. A 15% random sample of 1807 uninfected and lightly infected (< 3000 fluke eggs/g) subjects and all subjects with higher intensities of infection were invited to undergo an ultrasound examination. Among the 78% of subjects who complied, 44 had evidence of cholangiocarcinoma without overt symptoms. In nine of these, the diagnosis was corroborated by endoscopic retrograde cholangiopancreatography; a further six who died before they could undergo the procedure or who declined it were strongly suspected to have cholangiocarcinoma. Thus, there was a total of 15 cases, seven in patients who died with jaundice and hepatomegaly in 1991–92. Among 410 uninfected subjects, one case occurred. The multivariate prevalence odds ratios, accounting for age, sex and district of residence, were 1.7 (95% CI, 0.2–16.3) for subjects with up to 1500 fluke eggs/g, 3.2 (0.4–30) for subjects with 1501–6000 eggs/g and 14 (1.7–119) for more heavily infected subjects.

2.3.2 *Clonorchis sinensis*

In a consecutive series of 1484 autopsies in a single hospital in Hong Kong during the period 1964–66, clonorchiasis was found on gross examination in 11 of 17 (65%) cases of cholangiocarcinoma and in 24 of 83 (29%) cases of hepatocellular carcinoma. The expected proportions infected, on the basis of the whole series and adjusted for age and sex, were 38 and 35%, respectively. [The odds ratios, adjusted for age and sex, calculated by the Working Group, were 3.1 (95% CI, 1.1–8.4) for cholangiocarcinoma and 0.73 (0.45–1.2) for hepatocellular carcinoma] (Gibson, 1971).

Kim *et al.* (1974) studied records of autopsy and surgical specimens from one hospital in an area of low prevalence of *C. sinensis* (Seoul) and one hospital in an area of high prevalence (Pusan) in the Republic of Korea during the period 1961–72. In the area of low prevalence, a total of 386 histologically proven cases of primary liver cancer were identified among 1447 subjects with liver disease, and in the area of high prevalence, there were 109 cases of primary liver cancer among 396 subjects with liver disease. *C. sinensis* infection was determined by examination of liver tissue or stool samples. Comparison of cases of liver cancer with subjects with liver disease in whom cancer was not found showed a weak positive association between the cancer and *C. sinensis* infection [odds ratio, 1.7; 95% CI, 1.2–2.3]. The corresponding odds ratio for cholangiocarcinoma, based on 54 cases, was [6.5 (95% CI, 3.7–12)] and that for hepatocellular cancer, based on 423 cases, was [1.2, 0.80–1.7].

In Pusan, Republic of Korea, one of the areas of highest prevalence of *C. sinensis* infection, the occurrence of clonorchiasis was determined in stool specimens from 206 of a consecutive series of 368 cases of primary liver carcinoma diagnosed mainly in two hospitals during the period 1963–74 (Chung & Lee, 1976). [The Working Group noted that as one of these hospitals had been included in the study of Kim *et al.* (1974), there is some overlap with that study.] The control series comprised 559 subjects admitted to these hospitals with diseases other than of the liver; again, the presence of clonorchiasis was determined from stool specimens [no further details]. The crude odds ratio for cholangiocarcinoma, based on 36 cases, was [6.0 (95% CI, 2.8–13)]; the odds ratio was unchanged after adjustment for age

and sex. The crude odds ratio for hepatocellular carcinoma, based on 170 cases, was 1.1 (95% CI, 0.65–1.7).

These studies are summarized in Table 5.

3. Studies of Cancer in Animals

3.1 Infection with *Opisthorchis viverrini* alone

Hamster: In a histopathological study, a group of 30 male Syrian golden hamsters, three to four weeks of age, were infected with 100 metacercariae of *O. viverrini* by intragastric intubation. A group of 18 untreated hamsters served as controls. Five treated and three control animals were killed at 3, 7, 15, 30, 45 and 154 days after infection. The early pathological changes consisted of an acute inflammatory reaction involving the second-order bile ducts and portal connective tissue as well as focal coagulation necrosis of the liver lobules. As the liver flukes developed into adults (after 28 days), they induced hyperplasia, 'adenomatous formation' of the bile-duct epithelium, ductular proliferation and multilobular cirrhosis (Bhamarapravati *et al.*, 1978). [The Working Group noted the short duration of the study in relation to the lifespan of the animals, as it is possible that tumours could have developed in the animals if they had been allowed to live.]

As part of combination experiments (see section 3.2), a control group of 50 male Syrian golden hamsters, six to eight weeks of age, was given 50 *O. viverrini* metacercariae intragastrically and followed for 76 weeks. No bile-duct carcinoma was found (Flavell & Lucas, 1982, 1983).

Other groups of hamsters administered *O. viverrini* metacercariae alone as controls in combination experiments also had no bile-duct tumours after observation periods ranging from 22 to 45 weeks (Thamavit *et al.*, 1978, 1987a,b, 1988a,b, 1992a,b, 1994). In a further study (Thamavit *et al.*, 1993), a group of 18 female Syrian golden hamsters, six to eight weeks of age, received 60 *O. viverrini* metacercariae by intragastric intubation; 15 females received no treatment. Ten treated animals developed cholangiofibrosis and two developed cholangiocarcinomas within 38 weeks. No tumour was observed among controls. The difference in tumour rate was not significant.

A total of 150 male and 150 female Syrian hamsters, six to eight weeks of age, were divided into four groups and were infected monthly for 10 months with 0 (20 males and 20 females), 13 (40 males and 40 females), 25 (40 males and 40 females) or 50 (50 males and 50 females) *O. viverrini* metacercariae per intragastric intubation. Animals were then maintained on basal diet until they were killed at the end of week 52. Ten monthly intragastric applications of 0, 13, 25 or 50 metacercariae resulted in pronounced proliferative and inflammatory lesions involving the first- and second-order ducts, in response to the presence of adult worms. Cholangiofibrosis was seen, but no neoplastic lesion was evident after one year (Thamavit *et al.*, 1995). [The Working Group noted the short duration of the study.]

Table 5. Case–control studies of the association between *Chlonorchis sinensis* infection and cholangiocarcinoma and hepatocellular carcinoma

Location	Period of study	Type of cancer	Cases		Controls		Method of assessing *O. sinensis* infection	RR	95% CI	Reference
			Method of ascertainment	No.	Definition	No.				
Hong Kong	1964–66	CCA HCC	Autopsy	17 83	Autopsied subjects without CCA or HCC	1384	Gross examination at autopsy	3.1[a] 0.73[a]	0.13–8.4 0.45–1.2	Gibson (1971)
Republic of Korea, Seoul and Pusan	1961–72	CCA HCC	Autopsy and surgery of subjects with liver disease	54 423	Subjects coming to autopsy or surgery with liver disease in whom cancer was not found	1348	Examination of liver tissue or stool samples	6.5 1.2	3.7–12 0.80–1.7	Kim *et al.* (1974)
Republic of Korea, Pusan	1963–74	CCA HCC	Consecutive series of patients diagnosed mainly in two hospitals	36 170	Subjects admitted to these hospitals with diseases other than of the liver	559	Examination of stool samples	6.0 1.1	2.8–13 0.65–1.7	Chung & Lee (1976)

Relative risks and 95% confidence intervals calculated by the Working Group. CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma. The two last studies partially overlap.

[a]Adjusted for age and sex

3.2 Infection with *Opisthorchis viverrini* in combination with administration of known carcinogens

3.2.1 N-*Nitrosodimethylamine*

Hamster: Male Syrian golden hamsters, aged three to four weeks, were divided into four groups: 18 animals served as untreated controls; 21 animals received 0.0025% [25 mg/L] *N*-nitrosodimethylamine (NDMA) in the drinking-water starting from seven to eight weeks of age; 18 animals were infected with 100 *O. viverrini* metacercariae by intragastric intubation; and 21 animals were infected with *O. viverrini* and, four weeks later (as soon as the parasitic eggs were detected in faeces), received NDMA in the drinking-water. NDMA treatment was discontinued after 10 weeks, and animals were killed eight weeks thereafter (at 23 weeks). All of the animals that received NDMA and were infected developed cholangiocarcinoma and cholangiofibrosis. No such tumour was observed in the group that received either NDMA or parasite alone [$p < 0.001$; Fisher exact test], although cholangiofibrosis was found in some NDMA-treated animals (Thamavit *et al.*, 1978).

A total of 130 male Syrian golden hamsters, six to eight weeks of age, were divided into three groups: 50 animals were infected with 50 *O. viverrini* metacercariae by intragastric intubation, followed 41 days later by a single oral dose of 1.6 mg NDMA; 30 animals received a single oral dose of 1.6 mg NDMA on day 41; and 50 animals were infected with 50 *O. viverrini* metacercariae. Animals were maintained for 70 weeks or were killed when moribund. Cholangiocarcinomas developed in 5/50 infected animals given NDMA at latent periods of 18, 21, 29 (two animals) and 42 weeks after NDMA treatment. No malignant bile-duct tumour was found in any of the hamsters given either NDMA or metacercariae alone, but benign cystic cholangiomas [numbers not specified] were found commonly in these animals (Flavell & Lucas, 1982). [The Working Group noted that the authors did not report cholangiofibrosis in any of the groups. They also noted the single treatment and small dose of the carcinogen.]

A total of 176 male Syrian golden hamsters, six to eight weeks of age, were divided into four groups: 50 animals were infected with 50 *O. viverrini* metacercariae by intragastric intubation, followed 41 days later by a single oral dose of 1.6 mg NDMA; 46 animals received a single oral dose of 1.6 mg NDMA, followed 96 h later by infection with 50 *O. viverrini* metacercariae; 30 animals received a single oral dose of 1.6 mg NDMA; and 50 animals were infected with 50 *O. viverrini* metacercariae. Animals were killed when in poor condition or at the end of the 490-day experimental period. Mortality was highest in infected animals that received NDMA. Cholangiocarcinomas were observed in 5/50 animals (10%) that were first infected and then received NDMA and in 9/46 animals (20%) that received NDMA and were then infected. The difference between these two groups was not significant [Fisher's exact test]. None of the animals given NDMA alone or only infected with parasites developed malignant bile-duct tumours. The mean tumour latency was 249 days (range, 124–346 days) for the group that was first infected and then received NDMA, and that for the group that first received NDMA and were then infected was 308 days (range, 184–393 days); the difference was not significant. Tumours were most frequently found in the right liver lobe, the lobe in the hamster that also contains the largest proportion of *O. viverrini* worms (Flavell & Lucas, 1983).

A total of 280 male Syrian golden hamsters, three to four weeks of age, were divided into four main groups: one remained untreated; others were infected with 12, 25, 50 or 100 *O. viverrini* metacercariae by intragastric intubation; further groups were administered NDMA at 3, 6 or 12 mg/L in the drinking-water at four to five weeks of age for 10 weeks; and others wre infected with 12, 25, 50 or 100 metacercariae two weeks before administration of NDMA at 3, 6 or 12 mg/L in the drinking-water for 10 weeks. All animals were then maintained on basal diet until the end of the experiment at week 40, at which time they were killed. Only 2/17 animals (12%) in the group that received NDMA at 12 mg/L had detectable cholangiocarcinomas. No neoplastic lesion was seen in those that received NDMA at 6 mg/L or 3 mg/L, in those only infected or in untreated controls. In contrast, significant increases in the incidence of cholangiocarcinomas were seen in animals given both NDMA and metacercariae: 14/15, 10/17, 13/19, 7/10 [$p < 0.01$; Fisher's exact test]; and cholangiofibrotic lesions were observed (Thamavit *et al.*, 1987a).

Nitrite and aminopyrine can form NDMA in the stomach under certain conditions. A total of 150 male Syrian hamsters, three to four weeks of age, were divided into eight groups: one group was untreated; a second received 0.1% sodium nitrite in the drinking-water; one received 0.1% aminopyrine in the drinking-water; one received sodium nitrite and aminopyrine in the drinking-water; one was infected with 100 *O. viverrini* metacercariae by a single intragastric intubation; one was similarly infected and four weeks later received sodium nitrite in the drinking-water for 8 or 10 weeks; one was infected and four weeks later received aminopyrine in the drinking-water for 8 or 10 weeks; and the last was infected and four weeks later received sodium nitrite and aminopyrine in the drinking-water for 8 or 10 weeks. Hamsters that received the eight-week drinking-water treatment were killed 12 weeks later, and animals that received the treatment for 10 weeks were killed 20 weeks later. Combined administration of nitrite and aminopyrine for 8–10 weeks resulted in development of two hepatocellular nodules, seven cholangiofibrotic lesions and three cholangiocellular carcinomas. Prior infection with *O. viverrini* metacercariae induced inflammatory and proliferative changes in the livers of infected hamsters and was associated with a significant increase in the incidences of hepatocellular nodules (8; $p < 0.05$), cholangiofibrosis (18; $p < 0.05$) and cholangiocarcinomas (14; $p < 0.01$) (Thamavit *et al.*, 1988a).

A total of 105 male Syrian hamsters, six to eight weeks of age, were divided into four groups: 50 animals received a single intraperitoneal injection of 20 mg/kg bw NDMA, followed 19 days later by infection with 80 *O. viverrini* metacercariae by single intragastric intubation; 25 animals received the intraperitoneal dose of NDMA only; 15 animals were infected with *O. viverrini* only; and 15 animals served as untreated controls. Hamsters were killed when they became moribund or at the end of the experiment at 45 weeks. Among the 43 animals treated with both NDMA and *O. viverrini*, 19 developed cholangiocarcinomas, 40 developed cholangiofibrosis, 15 developed mucinous cystadenomas, 2 developed hepatocellular carcinomas and 42 developed hepatocellular nodules. Although 17/20 (85%) of the hamsters treated with NDMA alone developed hepatocellular nodules, with an average of 3.0 nodules per animal, there was an average of 9.5 nodules per animal in the combined treatment group. No lesion was observed in untreated controls, and 2/15 animals only infected with the parasite developed cholangiofibrosis. The difference in incidence of

cholangiocarcinomas between the combined group (19/45) and the group only infected with *O. viverrini* (0/20) was significant ($p < 0.001$; Fisher's exact test) (Thamavit *et al.*, 1994).

3.2.2 N-*Nitrosodiethylamine*

Hamster: A total of 180 female Syrian hamsters, three to four weeks of age, were divided into eight groups: 20 animals served as untreated controls; 20 animals were infected by gastric intubation with 60 *O. viverrini* metacercariae only; groups of 20–30 animals were infected with 60 *O. viverrini* metacercariae, followed four weeks later by administration of 10, 20 or 40 mg/L *N*-nitrosodiethylamine (NDEA) in the drinking-water for 12 weeks; and groups of 20–25 animals were administered only 10, 20 or 40 mg/L NDEA in the drinking-water for 12 weeks. The animals were killed at week 32. Infection with 60 metacercariae four weeks before administration of 20 or 40 mg/L NDEA resulted in significantly ($p < 0.01$) increased incidences of hepatocellular nodules in the groups also receiving NDEA (12/19 and 23/25, with 2.5 and 7.1 nodules/animal) when compared with the groups that received NDEA alone (3/19 and 9/21 with 0.2 and 0.9 nodules/animal). A high incidence of cholangiofibrosis was seen in animals receiving the combined treatment (Thamavit *et al.*, 1987b).

In a further study, 95 female Syrian golden hamsters, six to eight weeks of age, were divided into five groups: a group of 20 animals received a single intraperitoneal injection of 150 mg/kg bw NDEA dissolved in saline, and two groups of 20 animals each received NDEA followed 18 days later by infection with 50 or 100 *O. viverrini* metacercariae by intragastric intubation; 20 animals received 100 metacercariae without prior treatment with NDEA, and 15 animals were untreated. The animals were killed at the end of week 41. Infection with either 50 or 100 metacercariae of *O. viverrini* after NDEA injection resulted in significantly ($p < 0.01$) enhanced incidences of hepatocellular nodules/animal: 4.3 and 6.8 *versus* 1.4 in animals treated with NDEA alone (Thamavit *et al.*, 1992a).

3.2.3 N-*Nitrosodihydroxydi*-n-*propylamine*

Hamster: A total of 75 male Syrian golden hamsters, three to four weeks of age, were divided into four groups: 25 animals were infected with 100 metacercariae of *O. viverrini* per animal by gastric intubation and two and four weeks later received intraperitoneal injections of 1000 mg/kg bw *N*-nitrosodihydroxydi-*n*-propylamine (NDHDPA); 20 animals were treated with NDHDPA alone; 15 animals were infected with *O. viverrini* alone; and 15 animals served as untreated controls. Animals were killed at week 22. In the group treated only with NDHDPA, 2/20 animals had basophilic hepatocellular foci. Among 19 animals receiving combined treatment with NDHDPA and *O. viverrini*, six developed cholangiocarcinomas [$p = 0.02$], 18 developed cholangiofibrosis [$p = 0.001$] and nine developed hepatocellular nodules [$p = 0.002$] [all Fisher's exact test]; all 19 had hepatocellular basophilic foci, and eight had atypical proliferation of the pancreatic duct. Two of 20 animals given NDHDPA alone had hepatocellular basophilic foci (Thamavit *et al.*, 1988b).

A total of 100 male Syrian hamsters, three to four weeks of age, were divided into four groups: 10 animals served as untreated controls; 20 animals were infected with 80 *O. viverrini* metacercariae by intragastric intubation; 30 animals received three intraperitoneal

injections of 500 mg/kg bw NDHDPA at weeks 16, 17 and 18; and 40 animals were infected with 80 *O. viverrini* and received similar NDHDPA treatment. Animals were maintained on basal diet until they were killed, at week 52, when they were examined histologically. Cholangiocarcinomas occurred in 8/16 animals in the combined treatment group and 0/16 in that receiving NDHDPA alone [$p = 0.001$; Fisher's exact test]. Liver foci were seen in 16/16 hamsters in the combined treatment group and in 14/16 of those given NDHDPA, but the group receiving the combined treatment had a significantly increased number of foci per cm^2 (23.4 ± 7.5 *versus* 3.5 ± 2.6; $p < 0.001$) (Moore *et al.*, 1991).

3.3 Infection with *Opisthorchis viverrini* in combination with administration of other modifying factors

Hamster: A total of 115 male Syrian golden hamsters, six to eight weeks of age, were divided into four groups: 50 animals received five administrations of 60–80 *O. viverrini* metacercariae by intragastric intubation at weeks 0, 8, 16, 24 and 32 and 300 mg/kg bw praziquantel suspended in corn oil five weeks after the time of each administration; 30 animals were given praziquantel alone; and 20 animals received parasites alone, each by the above schedule; 15 animals served as untreated controls. Many of the animals infected with *O. viverrini* metacercariae became moribund and died (16/50 in the combined group; 8/20 in the group receiving infection alone). Surviving animals were killed at the end of week 40. Of the 34 surviving hamsters that received the combined treatment, one developed a cholangiocarcinoma, seven had cholangiofibrosis and one had a hepatocellular nodule. No such lesions were found in hamsters that received the drug alone, but 6/12 surviving hamsters that received infection alone developed cholangiofibrosis (Thamavit *et al.*, 1992b). [The Working Group noted the high mortality in the groups administered *O. viverrini* and the large total number of metacercariae administered.]

A total of 205 female Syrian golden hamsters, six to eight weeks old, were divided into seven groups of 25–40 animals each: three groups received two intraperitoneal injections of 1000 mg/kg bw NDHDPA dissolved in saline at two-week intervals; two weeks later, they were infected with 60 *O. viverrini* metacercariae by intragastric intubation and, at 4, 12 or 20 weeks, received a single dose of 250 mg/kg bw praziquantel suspended in corn oil by intragastric intubation. Two further groups received NDHDPA and *O. viverrini* by the same schedule, but with no praziquantel. One group received injections of saline at two-week intervals, followed two weeks later by infection with *O. viverrini*; another received the saline injections alone. The animals were maintained on basal diet and killed at the end of week 38. Of infected animals given NDHDPA, 16/16 developed cholangiofibrosis, 8/16 developed cholangiocarcinomas (2/18 in the group treated only with *O. viverrini* [$p = 0.015$; Fisher's exact test]) and 16/16 developed hepatic nodules with a multiplicity of 13.6 nodules/cm^2. Praziquantel administration at 4 or 12 weeks reduced the incidences of cholangiocarcinoma to 4/22 and 6/22, respectively. Praziquantal also reduced the multiplicity but not the incidence of hepatocellular nodules (3.6 nodules/cm^2 and 7.4 nodules/cm^2, respectively), but one animal in each of these groups also had a hepatocellular carcinoma. Cholangiofibrosis occurred in all animals treated with NDHDPA and *O. viverrini* plus praziquantel, except in those treated four weeks after infection, of which only 8/22 had cholangiofibrosis (Thamavit *et al.*, 1993).

3.4 Infection with *Opisthorchis felineus*

No data were available to the Working Group.

3.5 Infection with *Clonorchis sinensis* alone

3.5.1 *Rat*

As part of a combination study (see section 3.6.1), a control group of 25 male Wistar albino rats, 8–10 weeks of age, was administered 50 *Clonorchis sinensis* metacercariae by intragastric intubation. A few hepatic necrotic foci and mild inflammatory cell changes were seen in animals from each group killed at 4, 8, 12, 16, 20, 24 and 28 weeks after infection. Neither bile-duct lesions nor liver tumours were observed (Park, 1989). [The Working Group noted the short duration of the study and the inadequate reporting.]

In a further combination study, a control group of 10 male Fischer 344 rats, six weeks old, were each infected with 60 *C. sinensis* metacercariae by intragastric intubation and killed after 40 weeks. The infected animals developed cholangiocellular lesions, including bile-duct proliferation, periductal inflammation, fibrosis with occasional mucinous metaplasia, particularly at the main duct, and extensive areas of ductular proliferation. No tumour was observed (Jang *et al.*, 1990). [The Working Group noted the short duration of the study.]

3.5.2 *Cat*

Three cases of cholangiocarcinoma associated with *C. sinensis* infection were reported in cats (*Felis catus*) (Hou, 1964). Two of the cases were found at necropsy in two approximately four-year-old, well-developed, well-nourished cats out of a total of 215 obtained at random. The two cats harboured 150 and 200 adult *C. sinensis* in the liver. The third case was also in a four-year-old cat, which was one of 26 infected experimentally by feeding a diet of fish (*Ctenopharyngodon idellus*, *Hypophthalmichthys nobilis* and *Mylopharyngodon aethiops*) flesh containing metacercarial cysts of *C. sinensis* for 28 feedings. The animal died of bronchopneumonia; 105 *C. sinensis* were recovered from the bile ducts. The authors reported that the histopathological features of cholangiocarcinoma in the three cats were similar to those of many forms of bile-duct cancer found in humans infected with *C. sinensis* (Hou, 1956).

3.5.3 *Dog*

Cholangiocarcinoma associated with *C. sinensis* infection was also reported in one well-developed, well-nourished eight-year-old female chow dog, which had suffered from abdominal enlargement for an unknown period before death (Hou, 1965a). The histopathological features of the cholangiocarcinoma were reported to be similar to those of a form of bile-duct cancer found in humans infected with *C. sinensis* (Hou, 1956).

3.6 Infection with *Clonorchis sinensis* in combination with administration of known carcinogens

3.6.1 *Aflatoxin B_1*

A total of 75 male Wistar albino rats, 8–10 weeks old were divided into three groups: 25 rats were fed aflatoxin B_1 at 1 mg/kg diet for 12 weeks; 25 rats were infected by

administration of 50 *C. sinensis* metacercariae by intragastric intubation; and 25 animals were infected with *C. sinensis* and fed aflatoxin B_1 in the diet concomitantly. Three rats from each group were killed at four-week intervals up to 28 weeks after the beginning of treatment. Well-differentiated hepatocellular carcinomas were detected in two of three rats given the combined treatment and alive at 28 weeks; such tumours were not seen in rats treated with aflatoxin B_1 alone and killed at the same intervals (Park, 1989). [The Working Group noted the inadequate reporting of the study and the small comparison groups in the serial killings.]

3.6.2 N-*Nitrosodimethylamine*

Rat: A total of 101 male Fischer 344 rats, six weeks of age, were divided into six groups: 20 animals were each infected with 60 *C. sinensis* metacercariae by single intragastric intubation four weeks before receiving NDMA at 25 mg/L in the drinking-water for eight weeks; 20 animals were infected with *C. sinensis* while receiving NDMA at 25 mg/L in the drinking-water for eight weeks; 20 animals were infected with *C. sinensis* one week after NDMA treatment; 19 animals received NDMA in the drinking-water alone for eight weeks; 10 animals were infected with *C. sinensis* alone; and 12 animals served as untreated controls. The animals were killed at week 40, and all were found to have heavy helminthic loads. Livers were examined immunohistochemically for foci of the placental form of glutathione *S*-transferase. Animals infected before NDMA administration had significantly ($p < 0.05$) increased numbers of foci. No such effect was seen when animals were infected with *C. sinensis* during or after exposure to NDMA (Jang *et al.*, 1990).

Hamster: A total of 48 Syrian golden hamsters [sex unspecified], three to four weeks old, were divided into four groups: 12 animals received NDMA at 15 mg/L in the drinking-water for eight weeks and were given 10 metacercariae of *C. sinensis* suspended in saline by intragastric intubation seven days after the beginning of NDMA administration; 12 animals received the NDMA treatment alone; 12 received the helminthic treatment alone; and 12 animals served as untreated controls. After 11 weeks, 6/8 (75%) infected animals given NDMA developed cholangiocarcinomas, 8/8 developed cholangiofibrosis and 8/8 developed cholangiofibroma. Of the 12 animals given NDMA alone, two developed cholangiofibrosis and cholangiofibroma; of those given the helminth alone, 5/12 developed cholangiofibrosis. No lesions were observed in the 12 untreated controls (Lee *et al.*, 1993).

A total of 90 Syrian golden hamsters [sex unspecified], weighing 50–60 g, were divided into six groups of 15 animals each: one group received NDMA at 15 mg/L in the drinking-water for four weeks, followed one week later by administration of 15 metacercariae of *C. sinensis* suspended in saline by intragastric intubation; five weeks later the animals received oral administrations of 200 mg/kg bw praziquantel daily for three days. Another group was similarly infected with *C. sinensis* metacercariae but was treated with praziquantel for three days before treatment with NDMA. A further group received concomitant administration of NDMA and infection with *C. sinensis*. One control group received NDMA and another was infected with the helminth only. A final group served as untreated controls. At the end of 13 weeks, the group that had received concomitant treatment with NDMA and *C. sinensis* had 11/15 cholangiocarcinomas, 3/15 cholangiofibromas and 1/15 cholangiofibroses. In the group infected one week after NDMA treatment and given praziquantel,

3/15 had cholangiocarcinomas, 3/15 had cholangiofibromas and 6/15 had cholangiofibroses. In the group with combined treatment but given praziquantel three days before NDMA, 11/15 animals developed cholangiofibroses. In the group given NDMA alone, 4/15 animals had cholangiofibroma and 5/15 had cholangiofibroses; and in the group receiving only helminthic infection, 12/15 animals developed cholangiofibroses. No cancerous or pre-cancerous lesion of the bile duct was found in the untreated control group (Lee *et al.*, 1994).

3.6.3 *2-Acetylaminofluorene*

Hamster: Groups of 50 and 60 female Syrian golden hamsters, 8–10 weeks old, received 0 or 40 *C. sinensis* metacercariae per animal orally and were fed diets containing 0.03% 2-acetylaminofluorene for 40 weeks. After this time, all surviving animals were fed normal diets without carcinogen. Small numbers of animals from both groups were killed every three to four weeks from 0 up to 54 weeks, at which time the experiment was terminated. In animals that lived beyond 25 weeks, the incidence of cholangiocarcinomas was significantly ($p < 0.05$) higher in the infected group (11/14 animals) than in the uninfected group (6/17 animals). Metastases to other organs were observed only in infected animals with cholangiocarcinomas. The first bile-duct tumours were noted at 25 weeks in the infected group and at 35 weeks in the uninfected group (Iida, 1985).

4. Other Data Relevant for Evaluation of Carcinogenicity and its Mechanisms

4.1 Pathology of infection

4.1.1 *Humans*

(*a*) *Opisthorchis viverrini*

Tansurat (1971) described the detailed pathological features of infection with *O. viverrini* on the basis of 70 autopsied cases in Thailand. In early infections, there was no epithelial hyperplasia or fibrous proliferation. In chronic infections, there was proliferation of epithelial cells with formation of glandular acini, similar to the adenomatous changes in clonorchiasis, and there were varying degrees of periductal fibrosis. Enlargement of the liver is observed in most cases of opisthorchiasis, especially in cases of massive infection. The weight of the liver in massive infections is more than double the normal (3000–3500 g); the maximal weight recorded was 4000 g.

The major microscopic changes (Riganti *et al.*, 1989) are confined to the large and medium-sized bile ducts where the flukes are harboured. The cellular infiltrates consist of lymphocytes, monocytes, eosinophils and some plasma cells. Dilatation of the bile ducts, hyperplasia, desquamation and proliferation of the bile-duct lining cells, glandular formation and fibrosis of the periductal connective tissue of the walls are the commonest features. The gross and microscopic characteristics of human opisthorchiasis in 22 adults and seven children were similar, and the pathological changes were well established within 7–15 years after *O. viverrini* infection; however, dilatation of the gall-bladder, chronic cholecystitis and carcinoma were found only in adults.

In chronic and heavy infections, various degrees of cellular infiltration are caused by superimposed bacterial infection. This may result in suppurative cholangitis, and the infection may extend into the parenchyma of the liver tissue, causing cholangiohepatitis with abscess formation. Of 70 cases of advanced opisthorchiasis seen at autopsy, 10 showed multiple abscesses in the liver. The abscesses varied in diameter, from 5 to 10 mm; some ruptured into the right pleural cavity, and in some infections the lower lobe of the right lung was involved (Prijyanonda & Tandhanand, 1961).

In heavy infections with *Opisthorchis*, adult parasites are always discovered in the gall-bladder, the common bile duct and the pancreatic duct (Pungpak *et al.*, 1985, 1987). As in the large and medium-sized bile ducts, the parasites give rise to chronic cholecystitis. When there is superimposed bacterial infection, empyema of the gall-bladder may result. No stone formation was seen, however, either in the bile ducts or in the gall-bladder in one series of 70 cases at autopsy (Tansurat, 1971) or in another series of 154 cases (Koompirochana *et al.*, 1978). This finding is in contrast to that seen for clonorchiasis, in which cholelithiasis is one of the most serious complications (Rim, 1986). A number of biliary tract abnormalities associated with moderate to heavy *O. viverrini* infection were demonstrated by ultrasonography (Elkins *et al.*, 1990; Mairiang *et al.*, 1992). According to Mairiang *et al.* (1993), abnormal findings seen at ultrasonography improved dramatically after treatment with praziquantel.

(*b*) *Opisthorchis felineus*

Hepatic lesions produced by *O. felineus* are similar to those caused by *O. viverrini*. In the course of their development, they initiate inflammatory and proliferative changes of the biliary epithelium, which continue after the worms have matured and are accompanied by fibrosis of the distal biliary ducts. If the infection is intensified by continued exposure, the pathological process may extend to the bile ducts and gall-bladder and result in cirrhosis. The degree of pathogenicity and clinical involvement depends largely on the number of parasites and the duration of infection. Usually, small numbers of worms do not cause serious damage and do not give rise to clinical signs. In the Russian Federation, many apparently healthy people have been found to be infected; however, their worm burden was light, with an average of no more than 200 eggs/g faeces (Bronshtein *et al.*, 1991). When several hundred or thousand worms are present, severe damage to the liver and pancreas can occur (Rim, 1982a).

Hyperplasia of the epithelium of the larger bile ducts with cholangitis is much commoner. Advanced hepatic cirrhosis is rare. Occasionally, carcinoma of the bile ducts or of the pancreas, with metastases into the epigastric lymph nodes, is responsible for death (Faust *et al.*, 1970).

(*c*) *Clonorchis sinensis*

Most of the information on the pathological manifestations of *C. sinensis* comes from Hou's (1955) study of 500 autopsy cases. The liver appears grossly normal in light infections, but in heavy infections there is localized dilatation of the slightly thickened peripheral bile ducts (which can be seen on the surface beneath Glisson's capsule as pale-blue or greenish-blue blobs) and some atrophy of the parenchymal cells. The dilatation of bile ducts

is invariably caused by obstruction of the common bile duct by a stone, a tumour or inflammatory stricture resulting from cholangitis. Under these circumstances, nearly all the medium-sized bile ducts are dilated and filled with clear or turbid bile, with or without worms.

The major microscopic findings in the early stage of clonorchiasis are periductal oedema and acute inflammatory cellular responses in the bile duct walls. The bile ducts show not only desquamation but also marked hyperplasia of epithelial cells. Subsequently, marked goblet-cell metaplasia of ductal epithelial cells is seen, and remarkable adenomatous hyperplasia appears in the mucosa. Periductal connective tissue is increased around the biliary passages and the portal tract. In the chronic stage of infection, the ductal tissue is gradually replaced by fibrous tissue (sometimes described as cholangiofibrosis), which causes thickening of the bile duct wall (Hou, 1955).

The microscopic changes vary with the intensity and duration of infection and the coexistence of bacterial infections. Without secondary bacterial infection, the genuine histological changes are usually represented by a characteristic adenomatous formation, periductal fibrosis and heavy eosinophilic infiltration. With secondary bacterial infection, however, biliary obstruction is common and is due to adenomatous proliferation, calculi and cholangitis (Hou, 1955).

Extrahepatic involvement is relatively common in *C. sinensis* infection. Hou and Pang (1964) reported that 19/300 clonorchiasis patients had pancreatic involvement; Chan and Teoh (1967) found *C. sinensis* in 24 of 64 cases seen at autopsy. Adult fluke invasion of the pancreatic ducts occurs most frequently in heavy infections, but the pathological changes are usually less extensive than those in the intrahepatic bile ducts. The flukes reside in the main pancreatic duct and its tributary ducts. The changes are similar to those seen in the hepatic lesions: namely, adenomatous hyperplasia of ductal epithelium and, sometimes, squamous metaplasia (Chen *et al.*, 1994).

One of the most characteristic complications of clonorchiasis is formation of calculi in the intrahepatic biliary passages. It is sometimes accompanied by suppurative cholangitis, cholecystitis and biliary abscesses or so-called cholangiohepatitis and, ultimately, cholangiocarcinoma (Rim, 1986). The occurrence of calculi in clonorchiasis is due to bile stagnation caused by mechanical obstruction and the presence of worms and ova, which become nuclei for hepatolithiasis. Intra- and extrahepatic bile-duct calculi are composed almost entirely of bilirubin carbonate. According to Chen *et al.* (1994), the formation of pigmented stones in clonorchiasis can be attributed to changes in the concentrations of bilirubin, cholesterol, phospholipids and bile acids and the activity of bacterial glucuronidase in bile stagnation caused by mechanical obstruction. An increase in bacterial glucuronidase activity following *Escherichia coli* infection and glycoprotein in the bile favours the formation of pigmented stones (Guo *et al.*, 1990).

With goblet-cell metaplasia of the bile-duct epithelium, the bile has a high content of mucin, which combines with the presence of the helminth and its ova in the bile duct to cause cholestasis and to furnish a favourable environment for secondary bacterial infection. The most frequent infection is with *E. coli*, which induces ascending cholangitis from the intestine. Chou *et al.* (1976) studied mucin from 17 cases of clonorchiasis-associated

cholangiocarcinoma seen at autopsy. Histochemically, the mucins were qualitatively similar to those secreted by normal and *C. sinensis*-infected bile ducts, but the concentration of carboxymucins was reduced and sulfomucins were absent or present in only trace amounts in the neoplastic epithelium. Sulfomucins were abundant, however, in the hyperplastic epithelium of patients with clonorchiasis. The authors concluded that sulfomucins are valuable in differentiating hyperplastic bile ducts from cholangiocarcinoma.

Acute suppurative cholangitis may be caused by blockage of extrahepatic biliary ducts by masses of dead worms. Gallstones and the results of inflammation by bacterial infection often cause recurrent pyogenic cholangitis (Hou, 1955; Ong, 1962; Teoh, 1963). In a study of 525 *Clonorchis*-infected patients, only three had egg-induced lesions: an eosinophilic granuloma in the gall-bladder, a giant-cell reaction in the liver and pulmonary embolism (Sun, 1984). Periductal egg granulomas are rarely found (Sun, 1980).

Morphological studies by many investigators in Hong Kong and the Republic of Korea (Hou, 1956; Chou & Gibson, 1970; Kim *et al.*, 1974) indicate that carcinomas usually arise in association with pre-existing epithelial changes, which vary from hyperplasia to dysplasia and adenomatous formation in the secondary intrahepatic bile ducts.

Human cholangiocarcinoma can be divided into two macroscopic types according to the site of involvement, peripheral (intrahepatic) and hilar (extrahepatic). The peripheral type has multicentric growth as seen most frequently in *Clonorchis*-related neoplasms in patients, all of whom had histories of recurrent pyogenic cholangitis (Parkin *et al.*, 1993).

Of 38 subjects from Hong Kong chronically infected with *C. sinensis*, only one patient with cholangiocarcinoma had cirrhosis, whereas all but one patient with hepatocellular carcinoma had cirrhosis (Purtilo, 1976).

4.1.2 *Experimental systems*

(*a*) *Opisthorchis viverrini*

The pathological changes seen in the livers of cats, rabbits, guinea-pigs, hamsters and albino rats, which are considered to be suitable hosts, are grossly similar to those seen in man. After metacercariae are fed to animals, they grow into adult worms in the liver within about 30 days. The size of the worms found differs with species and is dependent on their size (Wykoff, 1958). Most studies of carcinogenesis have been conducted in Syrian hamsters, as the other species do not develop cholangiocarcinoma.

Bhamarapravati *et al.* (1978) described the histopathological response of Syrian hamsters to *O. viverrini* infection. The early changes consisted of an acute inflammatory reaction involving the second-order bile ducts and partial flattening of the epithelial cells, especially those in contact with the flukes. The main finding was foci of varying size consisting of liver cells that had undergone haemorrhagic and coagulation necrosis. Some multinucleated, foreign body-type giant cells were seen at the edge of the necrotic areas, but flukes were not found in these foci. The inflammatory reaction in the early stage of infection was predominantly eosinophilic infiltration of the portal areas, with some neutrophils and mononuclear cells. The dilated ducts showed hyperplasia and an atypical epithelial lining, which was piled up in places. An increase in the number of goblet cells was also evident. As the flukes developed into adults, they induced hyperplasia and adenomatous formations of

the bile-duct epithelium. There was also a granulomatous response to adult flukes and eggs. Resolution of the granulomas around eggs led to periductal and portal scarring and fibrosis. The major findings were two types of granuloma—one in response to the dying adult flukes and the other to the eggs. Dead or dying worms lying in bile ducts were surrounded by a granulomatous mass which consisted of eosinophilic, homogeneous, foamy material and various numbers of neutrophils, eosinophils and foamy macrophages. Granulomatous masses in the lumina of the ducts were usually connected to granulomatous masses in the periductal tissue through ulcerated areas of the mucosa. Numerous epithelioid granulomas containing eggs were seen in the periductal areas, occasionally extending into the lumen through the mucosal ulcers to connect with other granulomatous masses. The centres of the granulomas consisted of homogeneous eosinophilic precipitates and necrotic cellular debris. The shells of the eggs in some of the granulomas had been ingested by multinucleated giant cells, and in some granulomas the eggs were calcified.

(*b*) *Opisthorchis felineus*

The presence of *O. felineus* causes irritation of the intrahepatic bile ducts and pancreatic ducts, leading to a catarrhal inflammation and desquamation of the epithelium (Soulsby, 1965). As seen in *O. viverrini* and *C. sinensis* infections, extensive hyperplasia of the biliary system, papillomatous and adenomatous changes in bile ducts, cystic dilatation, necrosis and secondary atrophy of the hepatic cells, and extensive fibrosis occur in experimental animals (Rim, 1982b).

Formation of granulomas in the walls of bile ducts around *O. felineus* eggs was observed at days 20–25 of experiments in Syrian hamsters (Zubov & Mukanov, 1976).

(*c*) *Clonorchis sinensis*

Many laboratory animals are sensitive to *C. sinensis*. Rabbits and guinea-pigs are the most susceptible; rats, Syrian hamsters and dogs are relatively susceptible; and mice are the least susceptible of these species. The degree of pathological change depends on both the intensity and the duration of infection. The major pathological findings in the livers of animals with clonorchiasis are in the biliary system, which the helminths inhabit. The most characteristic pathological change in infection is diffuse adenomatous tissue formation in the secondary bile ducts. Desquamation, hyperplasia of lining epithelial cells, regeneration and adenomatous hyperplasia are seen (Hou, 1965b; Kim *et al.*, 1974).

Microscopically, periductal and ductal aggregations of inflammatory cell infiltrates are usually profound in the acute stage and consist of lymphocytes, plasma cells, histiocytes and fibrosis. Hyperplasia of epithelial cells is frequent (Rim, 1982b). Small eosinophilic abscesses and focal liver cell necrosis may be present, but the hepatic lobular structure remains intact (Chen *et al.*, 1994).

Cha *et al.* (1991) noted in rats infected repeatedly with *C. sinensis* that a heavy eosinophilic infiltration appeared around the bile duct after two to four weeks. The cells were then replaced by massive mononuclear cells, which often formed lymphoid follicles. In similarly infected mice, the epithelial cells of the bile duct were changed to secretory cells, which secreted hyalinized materials into the lumen of the bile duct. Inflammatory cells infiltrated the adjacent hepatic parenchyma and formed microabscesses.

Clonorchis infection induces severe hyperplasia of epithelial cells and metaplasia of mucopolysaccharide producing cells in the biliary epithelium (Lee, S.H. *et al.*, 1978; Song *et al.*, 1989; Hong *et al.*, 1990). In a study of the proliferative activity of bile-duct epithelial cells in clonorchiasis by immunostaining bromodeoxyuridine incorporated into the DNA of cells in the S phase of division (Risio *et al.*, 1988), the greatest rate was found mainly in cells located at the base of the mucosal layer (Hong *et al.*, 1993). The authors suggested that mucosal epithelial cells of bile ducts infected with *C. sinensis* become hyperplastic mainly by direct and local stimulation by the worms.

Hepatic changes in rabbits in the early stage (first two weeks) of infection were reversible after treatment with praziquantel; however, some of the biliary epithelial changes that occurred in the chronic stage (12 weeks) of infection were irreversible (Lee *et al.*, 1989).

In guinea-pigs infected with *C. sinensis*, the biliary epithelium had an increased prevalence of mucin granules, cytoplasmic projection into the lumen, decreased numbers of microvilli and obstruction of the bile canaliculi. Blurring or irregularity of intercellular lateral interdigitation was observed in most of the biliary epithelium. Hepatocytes showed dilatation of endoplasmic reticulum and destruction of cristae in some mitochondria (Lee, Y.S. *et al.*, 1978).

4.1.3 *Comparison of humans and experimental animals*

The general pathological features of clonorchiasis and opisthorchiasis are similar in human cases and experimental animals, including the Syrian hamster, which is the most commonly used species in carcinogenicity studies. The changes involve predominantly the intrahepatic bile ducts and pancreatic ducts. The initial changes, such as desquamation of the bile-duct lining cells, are followed by hyperplasia of the cells lining the intrahepatic bile ducts and are identical in humans and in the acute stage of experimental infections. In chronic infections in humans and experimental animals, adenomatous hyperplasia of the bile ducts, heavy eosinophilic infiltration and periductal fibrosis occur. Secondary bacterial infections, especially ascending infection with *E. coli*, result in multiple hepatic abscesses and cholangiohepatitis in livers infected by both *Opisthorchis* and *Clonorchis*.

4.2 Other observations relevant to the interpretation of carcinogenicity and mechanisms of carcinogenesis

4.2.1 *Humans*

Inflammatory responses in host tissues challenged by infections or inflammatory agents have been postulated to play a role in the development of cancers which arise in infected organisms (for reviews, see Gentile & Gentile, 1994; Ohshima & Bartsch, 1994). Reactive oxygen species and nitrates, nitrites and various nitrosating agents are produced to kill invading microorganisms and helminths. Polymorphonuclear leukocytes play a prominent role in the production of these host defence agents (for reviews, see Preussmann & Eisenbrand, 1984; Shepard *et al.*, 1987). The radicals have been shown to induce genetic damage in normal host tissues adjacent to the site of inflammation, producing DNA strand breaks, mutations and chromosomal aberrations (Weitzman & Stossel, 1981; Birnboim,

1982). While no data on humans are available to verify these observations, increased levels of urinary nitrates and salivary nitrites are found in *O. viverrini*-infected individuals.

Srianujata *et al.* (1984) reported significantly higher concentrations of nitrate (2–2.8 times) and nitrite (2–5.6 times) in the saliva of inhabitants in a high-risk area for cholangiocarcinoma in North-east Thailand than in subjects in Bangkok (low-risk area). Nitrate levels in urine were also significantly higher (1.5–3 times) in the subjects from the high-risk areas. Srianujata *et al.* (1987) also reported higher levels of nitrite (1.8 times) and *N*-nitrosoproline (2.6 times) in the urine of subjects infected with *O. viverrini* than in uninfected subjects from the same area of North-east Thailand. Haswell-Elkins *et al.* (1994b) confirmed these observations in a study in North-east Thailand in which diet and smoking were controlled for; they also demonstrated decreased concentrations of nitrates and nitrites in these subjects after treatment with praziquantel. Srivatanakul *et al.* (1991c), in a study in which diet and smoking were not controlled for, reported that subjects living in high-risk areas for fluke infection who had antibodies to *O. viverrini* had a 10-fold greater potential for endogenous nitrosation, measured on the basis of urinary levels of *N*-nitrosoproline after proline ingestion, than individuals who did not have antibodies.

Cholangiocarcinomas from *O. viverrini*-infected patients differed from those in uninfected patients with respect to point mutations in the c-Ki-*ras* proto-oncogene: mutations were found at codon 12 of this gene in five of nine individuals in Japan who had cholangiocarcinoma but no concomitant fluke infection, but not in six patients from Thailand who harboured both cholangiocarcinoma and fluke infection (Tsuda *et al.*, 1992). Similar results were reported by Kiba *et al.* (1993), who found, however, that a mutation at the *p53* tumour suppressor gene was similar in the two sets of cholangiocarcinoma patients, all but one being GC→AT transitions in a highly conserved GpG site.

4.2.2 *Experimental systems*

In male Syrian hamsters and jirds (*Meriones unguiculatus*), 220 days after experimental infection with *O. viverrini*, marked proliferation of smooth endoplasmic reticulum was observed in hepatocytes, and lobed and enlarged nuclei and mitochondria were seen which showed significant pathological degeneration, up to lysis. There was also accumulation of intermediate filaments in adjacent bile-duct epithelia and in the epithelium of the gall-bladder (Adam *et al.*, 1993). Depressed lymphoproliferative response to phytohaemagglutinin stimulation has also been described in Syrian hamsters infected with *O. viverrini*, suggesting an immunodepressive effect (Wongratanacheewin *et al.*, 1987).

The role of *O. viverrini* in enhancing host response to chemical carcinogens (particularly nitrosamines) has been well documented in Syrian hamster models (see section 3.2). A significant increase in the proportion of water-soluble aflatoxin B_1 metabolites was found in hamsters infected with liver flukes over that measured in uninfected animals (Makarananda *et al.*, 1991), suggesting increased expression of enzymes that metabolize aflatoxin B_1. A cytochrome P450 isozyme(s) (CYP2A) has been identified in the livers of hamsters infected with *O. viverrini*, the activity of which increased nonuniformly in male but not female animals, the highest levels of activity occurring in hepatocytes immediately adjacent to areas of inflammation. This increase occurred in spite of a decrease in the total hepatic P450 content. The enzyme was shown to contribute up to 50–60% of the metabolism

of hepatic aflatoxin B_1 and *N*-nitrosodiethylamine in infected males and 20–40% in infected females (Kirby *et al.*, 1994).

Immunohistochemical analysis of aflatoxin B_1–DNA adducts in parasitized animals indicated that the greatest numbers of adducts occurred in the regions of highest CYP2A activity. Studies with a related liver fluke, *Fasciola hepatica*, also showed enhanced cytochrome P450-related activation of aflatoxin B_1 into a mutagen by liver extracts from fluke-infected mice over that with extracts prepared from livers of uninfected animals (Gentile & DeRuiter, 1981).

Nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase was found to be increased in *O. viverrini*-infected Syrian hamsters, and nitric oxide synthase activity in liver cytosol was twice as high in infected as in untreated hamsters The enzyme was located in macrophages and eosinophils which accumulated at the site of the infection (Ohshima *et al.*, 1994).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

The liver flukes, *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*, are biologically similar, food-borne trematodes which chronically infect the bile ducts and, more rarely, the pancreatic duct and gall-bladder of human beings and other mammals. Infection is acquired by eating raw or undercooked freshwater fish which contain the infective stage (metacercaria) of flukes. Immature flukes migrate up through the ampulla of Vater to the biliary tree, mature in the small intrahepatic ducts and produce eggs, which are passed in the faeces. If the eggs reach a water body and are consumed by an appropriate species of snail, they hatch and undergo asexual multiplication to produce free-swimming larvae, which can penetrate freshwater fish and become encysted metacercariae.

Liver fluke infections are best detected by identification of eggs in the faeces. In light infections and severe disease with obstruction, eggs may not be found. There is a close quantitative relationship between the number of eggs per gram of faeces and the number of adult worms. Immunodiagnostic techniques cannot be used reliably to detect active infections.

Nine million people are infected with *O. viverrini*, which is common in North-east Thailand, at least one-third of the population being infected, and in North Thailand and Laos. *O. felineus* affects 1.5 million people, mainly in the central part of the Russian Federation. An estimated 7 million people are infected with *C. sinensis* in the Republic of Korea, southern China, Hong Kong, Macao and Viet Nam. The distribution of human infection is determined primarily by the distribution of the habit of eating raw freshwater fish; heterogeneity within endemic areas is probably due to environmental factors and control. Infection generally occurs during the first decade of life, often with a similar pattern in men and women, although men may be more frequently and heavily infected than women.

Most liver fluke infections lead to local inflammation, and they are associated with specific clinical signs and symptoms in 5–10% of infected people. The intensity of infection is

correlated with hepatobiliary tract abnormalities visualized by ultrasound. Biliary and gall-bladder stones are commoner among individuals heavily infected with *Clonorchis* than among those infected with the other liver flukes. Treatment with praziquantel is highly effective and also leads to reversal of biliary tract abnormalities. Control of infection has been achieved in some areas by a combination of chemotherapy, health education and improved sanitation.

5.2 Human carcinogenicity data

Opisthorchis viverrini

Within Thailand, the highest proportional incidence rate of cholangiocarcinoma is observed in the north-east region of the country where the prevalence of infection with *O. viverrini* is also highest. In this region, the incidence of cholangiocarcinoma is about 40 times the highest incidence outside Thailand. A formal analysis across five regions of the country showed a strong correlation between proportional incidence of cholangiocarcinoma and estimated average titres of antibodies to *O. viverrini* and, to a lesser degree, faecal egg count. Correlations with proportional incidence rates of hepatocellular carcinoma were much weaker.

Many cases of liver cancer arising in patients with *O. viverrini* infection have been reported from Thailand. In most regions of the world, cholangiocarcinoma is a very rare tumour. In areas where *O. viverrini* is endemic, however, the numbers of cases of cholangiocarcinoma generally outnumber those of hepatocellular carcinoma.

Three cross-sectional or case–control studies of the association between infection with *O. viverrini* and cancer of the liver have been reported from Thailand. In the earliest and smallest of these studies, the estimated relative risks for cholangiocarcinoma and hepatocellular carcinoma in association with the presence of *O. viverrini* eggs in faeces were each 1.3. In the second study, the estimated relative risk for the association between cholangiocarcinoma and the presence of *O. viverrini* antibodies in serum was 5.0, which was significant. The association was not explained by possible confounding with hepatitis B virus infection or estimated recent intake of aflatoxins. The estimated relative risk for the association with hepatocellular carcinoma was 1.7 (not significant). In the third study, based on 15 cases of cholangiocarcinoma, estimated relative risks of 1.7, 3.2 and 14.1 were calculated for categories of faecal excretion of increasing numbers of *O. viverrini* eggs. This trend was highly significant.

Opisthorchis felineus

The incidence of liver cancer was observed to be correlated with the prevalence of infection with *O. felineus* across four areas in the T'umen' region of north-west Siberia. Cases of both cholangiocarcinoma and hepatocellular carcinoma have been reported in people infected with *O. felineus*.

Clonorchis sinensis

Cases of cancer of the liver in association with infection with *C. sinensis* have been reported from China, Hong Kong, the Republic of Korea and Japan and in immigrants to North America from China and Laos.

Two case–control studies of the relationship between *C. sinensis* infection and liver cancer, with partially overlapping case series, have been carried out in the Republic of Korea. Significantly increased estimated relative risks of 6.5 and 6.0 were seen for an association with cholangiocarcinoma, but no significant association was seen with the occurrence of hepatocellular carcinoma. In a third case–control study, in Hong Kong, the estimated relative risk for cholangiocarcinoma, after adjustment for age and sex, was 3.1, while that for hepatocellular carcinoma was 0.7.

5.3 Animal carcinogenicity data

Infection with *O. viverrini* alone was evaluated in hamsters in several studies that were not designed specifically as long-term carcinogenicity studies. Two cholangiocarcinomas were found in one of these studies. In several studies in hamsters infected with *O. viverrini* and treated with various carcinogenic *N*-nitrosamines, induction of cholangiocarcinomas and of hepatocellular nodules was enhanced.

No study of the carcinogenicity of *O. felineus* was available.

Infection with *C. sinensis* was associated with the presence of a few cholangiocarcinomas in cats and one in a dog. Two experiments in rats were inadequate for evaluation. Infection with *C. sinensis* increased the incidence of cholangiocarcinomas in hamsters treated with 2-acetylaminofluorene or *N*-nitrosodimethylamine.

5.4 Other relevant data

The general pathological features of infection with liver flukes are similar in humans and animals. The changes are characterized by oedema, desquamation and acute inflammatory cellular responses in the bile ducts in the early stage; in the chronic stage, the bile ducts show marked goblet-cell metaplasia, adenomatous hyperplasia and thickening of the walls. Complications may include calculi, suppurative cholangitis and biliary abscess caused by bile stagnation due to mechanical obstruction.

Cholangiocarcinomas appear to arise from pre-existing adenomatous changes in the bile ducts through the phase of intestinal metaplasia or dysplastic change.

The expression of CYP2A isozymes that catalyse the metabolism of aflatoxin and nitrosamines in the liver is increased in *O. viverrini*-infected hamsters. The increased expression is located in regions of the liver adjacent to the site of inflammation. The activity of macrophage-associated nitric oxide synthase is also increased in these animals. No information was available about the effects of liver fluke infection on carcinogen metabolism in humans. Increased urinary levels of nitrate and certain nitrosamines are detected in people infected with *O. viverrini*.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *inadequate evidence* in humans for the carcinogenicity of infection with *Opisthorchis felineus*.

There is *limited evidence* in humans for the carcinogenicity of infection with *Clonorchis sinensis*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *inadequate evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis felineus*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Clonorchis sinensis*.

In making the overall evaluation, the Working Group noted that experimental and epidemiological studies on *Clonorchis sinensis* confirm:

(i) that the biological and pathological characteristics of *Opisthorchis* and *Clonorchis* are similar;

(ii) that cholangiocarcinoma occurs in infected animals, especially when infection is combined with administration of known carcinogens; and

(iii) that the relative risks for cholangiocarcinoma, and not for hepatocellular carcinoma, are consistently increased in people infected with this organism.

Overall evaluations

Infection with *Opisthorchis viverrini* is carcinogenic to humans *(Group 1)*.

Infection with *Opisthorchis felineus* is not classifiable as to its carcinogenicity to humans (Group 3).

Infection with *Clonorchis sinensis* is probably carcinogenic to humans (Group 2A).

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INFECTION WITH *HELICOBACTER PYLORI*

1. Exposure Data

1.1 Structure and biology of *Helicobacter pylori*

1.1.1 *Taxonomy*

The presence of spiral-shaped bacteria on human gastric mucosa was first recognized nearly one hundred years ago (Pel, 1899). These bacteria were isolated for the first time in 1982, in cultures of endoscopic biopsy specimens from patients with gastritis and peptic ulceration (Marshall, 1983; Warren, 1983). For phenotypic reasons, such as spiral shape, motility, growth under microaerophilic conditions and isolation from the alimentary tract, the organism was classified as a member of the genus *Campylobacter* and was called *Campylobacter pyloridis* (Marshall *et al.*, 1987), and then *C. pylori* (Marshall & Goodwin, 1987). It became clear, however, that *C. pylori* differed significantly from other members of the genus with respect to cellular fatty acids, lack of a methylated menaquinone, antimicrobial susceptibility and ribosomal ribonucleic acid sequences.

In 1989, a new genus, *Helicobacter*, was proposed, and *C. pylori* was renamed *Helicobacter pylori* (Goodwin *et al.*, 1989). The genus now includes a variety of 'gastric' and 'non-gastric' *Helicobacter* species. Classification of bacteria into the new genus was based mainly on a homology greater than 90% of the nucleotide sequence in the 16S ribosomal RNA molecule (Lee & O'Rourke, 1993). The gastric *Helicobacter* spp. are *H. pylori*, *H. mustelae* (ferrets; Fox *et al.*, 1986, 1988), *H. felis* (cats and dogs; Lee *et al.*, 1988, 1990, 1992), *H. nemestrinae* (macaque monkeys; Bronsdon *et al.*, 1991) and *H. acinonyx* (cheetahs; Eaton *et al.*, 1991a). One non-gastric *Helicobacter* sp. is *H. hepaticus* (mouse liver and intestine; Fox *et al.*, 1994). An additional spiral bacterium commonly found in the stomachs of cats, dogs and pigs and infrequently in those of humans, which has not yet been cultured and is known provisionally as '*Gastrospirillum hominis*' or '*H. heilmannii*', has been proposed for addition to the genus on the basis of morphological and RNA similarities (Solnick *et al.*, 1993).

1.1.2 *Biology*

(a) Morphology; ultrastructural features

H. pylori is a spiral or slightly curved gram-negative rod with two to six characteristic unipolar flagella. The bacterium has bluntly rounded ends and measures 2.5–4.0 μm in length and 0.5–1.0 μm in width. The cell wall is smooth and may be coated with a prominent

glycocalyx with a thickness up to 40 nm (Goodwin *et al.*, 1989); it is covered with ring-like subunits with a diameter of 12–15 nm. Occasionally, bacteria may contain bacteriophages. The flagella measure 2.5 μm in length and around 30 nm in thickness and have a distinctive terminal bulb (Goodwin & Worsley, 1993). Each flagellum consists of a central filament enveloped by a flagellar sheath. The filament consists mainly of a polymer of a 53-kDa [80 base-pair] flagellin protein (Geis *et al.*, 1989, 1993); it ends proximally in a basal body, which is associated with the cytoplasmic membrane. The sheath is formed by a lipid bilayer, which extends as a direct continuation from the bacterial outer membrane (Geis *et al.*, 1993). The bacterium displays remarkable motility in viscous solutions, and the flagella play a central role in this motility (Hazell *et al.*, 1986; Suerbaum *et al.*, 1993). *H. pylori* may change from its normal morphological appearence into a range of coccoidal forms, especially *in vitro* after prolonged culture or after antibiotic treatment. It is not certain whether the coccoidal forms can resume the spiral, multiplying form. The viability of coccoidal organisms has been proven by means of acridine orange staining, bromodeoxyuridine incorporation and urease activity (Goodwin & Worsley, 1993; Nilius *et al.*, 1993).

(*b*) *DNA content; genome and plasmids*

The DNA of different *H. pylori* strains contains 34–38 mol % guanine and cytosine (Goodwin & Worsley, 1993). The genome varies in size from 1.6 to 1.73 megabases (Taylor *et al.*, 1992). About 35–50% of *H. pylori* strains contain plasmids, which have not been associated with any biological characteristic of the bacteria (Majewski & Goodwin, 1988; Penfold *et al.*, 1988; Simor *et al.*, 1990).

A number of specific genes have been cloned, including two structural urease genes which encode the subunits of the urease enzyme (Labigne *et al.*, 1991), two flagellin genes, called *fla*A (Leying *et al.*, 1992) and *fla*B (Suerbaum *et al.*, 1993), a cytotoxin production-associated gene, the *cag*A gene (Tummuru *et al.*, 1993), the cytotoxic *vac*A gene (Cover *et al.*, 1994) and a heat-shock protein encoding gene (Macchia *et al.*, 1993).

(*c*) *Growth conditions*

H. pylori can be cultured in both solid and liquid media. Basal solid media, such as Colombia blood agar base and brain–heart infusion agar supplemented with serum or charcoal, yield good results (Dent & McNulty, 1988; Goodwin & Worsley, 1993). Brain–heart infusion (or brucella) broth supplemented with charcoal, serum or cyclodextrins can also be used (Olivieri *et al.*, 1993). Microaerophilic culture conditions are essential, with optimal oxygen concentrations between 2 and 8%. Addition of extra carbon dioxide or 1–5% whole blood or serum may stimulate culture yields. Bacteria of the genus *Helicobacter* do not catabolize carbohydrates (Mégraud *et al.*, 1985; Goodwin & Worsley, 1993), but *H. pylori* can use glucose via the pentose phosphate pathway (Mendz *et al.*, 1993). Maximal growth occurs at 37 °C and neutral pH (Goodwin & Worsley, 1993). The bacterium is sensitive to almost all antibiotics *in vitro*, with the exception of nalidixic acid, trimethoprim, sulfonamides and vancomycin (Goodwin *et al.*, 1989; Goodwin & Worsley, 1993). Section 1.5 provides further information about the efficacy of antibiotics *in vivo*.

(*d*) *Enzymatic activity*

H. pylori is characterized by strong urease activity, with a Michaelis constant of 0.48 mmol/L for urea (Goodwin & Worsley, 1993). The hexameric enzyme has a relative molecular mass of about 600 kDa [909 base pairs] and is composed of six monomers, each with two protein subunits of 66 and 31 kDa [100 and 47 base pairs]. It is active at pH 4.0–10.0 and has an isoelectric point of 5.93 (Evans et al., 1992; Goodwin & Worsley, 1993; Mobley & Foxall, 1994). Of the total protein production of the bacterium, 6% consists of urease (Hu & Mobley, 1990). The urease molecule is associated with a 62-kDa [94-base-pair] heat-shock protein, the function of which has not been fully elucidated (Evans *et al.*, 1992).

H. pylori is oxidase-positive and produces large amounts of catalase (Goodwin *et al.*, 1989) and superoxide dismutase (Spiegelhalder *et al.*, 1993). The tetrameric catalase, with subunits of 50 kDa [76 base pairs], has an isoelectric point of 9.0–9.3. *H. pylori* also produces phospholipase A2 and C, γ-glutamyltranspeptidase, DNase, both acid and alkaline phosphatase, a mucus-degrading glycosulfatase (Mégraud *et al.*, 1985; Freland & Drugeon, 1988; Slomiany *et al.*, 1992; Ottlecz *et al.*, 1993), alcohol dehydrogenase (Salmela *et al.*, 1993) and leucine aminopeptidase (Mégraud *et al.*, 1985). It has significant alcohol dehydrogenase activity at both low and high concentrations of ethanol (Salmela *et al.*, 1993; Salaspuro, 1994). Hippurate hydrolysis and nitrate reduction do not occur (Goodwin & Worsley, 1993), nor does *H. pylori* contain indole or produce hydrogen sulfide (Mégraud *et al.*, 1985).

1.1.3 *Agent–host relationship*

(*a*) *Host and target tissues*

Natural infection with *H. pylori* has been demonstrated only in humans and in nonhuman primates. Oral challenge under laboratory conditions may lead to colonization in *Macaca* species, gnotobiotic piglets and dogs (Fox *et al.*, 1991). The reasons for this narrow host range are unknown but may be related to specific binding capacities for human mucosal antigens (Husson *et al.*, 1993). In infected humans, *H. pylori* specifically colonizes the gastric mucosa, as it is uniquely adapted to survive the acidic environment. Within the stomach, infection is usually greatest in the antrum (Dixon, 1991); colonization densities in the acid-producing corpus region of the stomach are lower. For unknown reasons, antral colonization may decrease and corpus colonization may increase under conditions of lower acid output (Louw *et al.*, 1993). Microscopically, the bacterium can usually be observed within the surface mucus layer, both on the surface epithelium and within the pits. Under the electron microscope, it is usually observed close to intercellular junctions of mucus-secreting cells (Hazell *et al.*, 1986; Caselli *et al.*, 1989). It is not found in areas of intestinal metaplasia (Correa *et al.*, 1989). Epithelial cell invasion is very rare (Caselli *et al.*, 1989). The specific affinity of *H. pylori* for gastric epithelium is exemplified by the occasional demonstration of these bacteria on metaplastic gastric mucosa in the oesophagus (Paull & Yardley, 1988), in the duodenum, in Meckel's diverticulum or in the rectum (Offerhaus *et al.*, 1990; Kestemberg *et al.*, 1993).

Interest in possible routes of transmission (see section 1.3) has focused research on the presence of *H. pylori* in the mouth and faeces of infected individuals. Although *H. pylori* has been detected in both dental plaque and faeces (Thomas *et al.*, 1992; Nguyen *et al.*, 1993), a limited number of successful isolations have been made, the number of cases studied is small,

and occasionally the cultured bacteria have been incompletely identified. The bacterium has been found only in the gastrointestinal tract.

(*b*) *Immune response of infected individuals*

The presence of *H. pylori* on the gastric mucosa elicits an inflammatory response in all infected individuals. This response is characterized by inflammatory cells in the mucosa (see sections 1.4 and 4.1) and by local and systemic humoral immune responses. The specific immunoglobulin (Ig)A response, both locally and systemically, consists mainly of the IgA1 subclass (van der Est *et al.*, 1992). The systemic IgG response involves all four subclasses. Different subclass responses have been noted in gastritis patients with and without duodenal ulcer; it is unknown whether this difference is related to the host or to the bacterial strain (Bontkes *et al.*, 1992). The IgG response diminishes within 6–12 months after the infection has been eradicated with antibiotics (Kosunen *et al.*, 1992). It also appears to diminish after histological disappearance of *H. pylori* due to the development of gastric mucosal atrophy, which is unfavourable to colonization; however, only retrospective evidence is available to substantiate this claim (Crabtree *et al.*, 1993a), and long-term follow-up studies have not yet been carried out (Kuipers *et al.*, 1994a).

(*c*) *Colonization factors*

A variety of factors play a role in the establishment and maintenance of *H. pylori* colonization in the strongly acidic stomach. Motility makes possible rapid transit through the acidic lumen and penetration into the viscous epithelial mucus layer, which protects against acid contact. The unipolar flagella are essential for this motility: aflagellated mutants have been shown to be immobile (Suerbaum *et al.*, 1993). Adherence to the gastric epithelium is the next important factor for virulence. Microscopic research has shown adherence to epithelial pedestals (Caselli *et al.*, 1989), and several investigators have shown specific binding capacities for both extracellular matrix proteins and cellular antigens (Borén *et al.*, 1993; Moran *et al.*, 1993). Binding to Lewis^b blood group antigens has been reported (Borén *et al.*, 1993).

The production of enzymes, especially urease, is a third factor of importance in *Helicobacter* colonization. In laboratory experiments, a mutant strain of *H. pylori* with only very weak urease activity was unable to colonize gnotobiotic piglets (Eaton *et al.*, 1991b). Urease inhibition does not, however, eradicate an established infection. *In vitro*, urease-positive bacteria do not survive at pH 1.5 in the absence of urea but can survive when urea is added (Marshall *et al.*, 1990; Ferrero & Lee, 1991). These observations led to the hypothesis that the potent urease is required to establish new infections; however, once the bacteria have reached a protected niche deep within the mucus layer, protection is no longer necessary and urease may be needed only for delivery of nitrogen.

(*d*) *Pathogenic mechanisms*

In the interaction between *H. pylori* and the gastric mucosa, a number of factors have been claimed to play a role in the chronic inflammatory reaction and epithelial cell damage which, in some cases, lead to overt clinical disease (see section 1.4). Firstly, the bacterium secretes several enzymes that can alter the integrity of both the mucus and epithelial cells. It

produces a glycosulfatase that causes loss of mucus viscosity and a diminished capacity to retard hydrogen ion diffusion (Slomiany *et al.*, 1992); mucus secretion is also diminished (Micots *et al.*, 1993). Ammonia produced by the potent urease enzyme is directly toxic to gastric epithelial cells both *in vivo* and *in vitro* (Mégraud *et al.*, 1992; Tsujii *et al.*, 1992). The phospholipase activity of the bacterium (Daw *et al.*, 1993) can cause degradation of membrane phospholipids, and its alcohol dehydrogenase activity leads to production of the toxic acetaldehyde in the presence of ethanol (Salmela *et al.*, 1993). The clinical importance of the latter finding is unknown.

Helicobacter also produce a variety of substances that may damage the infected host. Shedding of bacterial surface proteins in close proximity to the mucosa may have a chemotactic action on leukocytes (Mai *et al.*, 1992). About 50–60% of *H. pylori* strains can produce a cytotoxic protein that causes vacuolization of cultured epithelial cells (Cover *et al.*, 1990; Fox *et al.*, 1992).

1.2 Methods for detection of infection

1.2.1 *Methods based on gastric biopsy specimens*

Specimens collected before treatment from both the antrum and the corpus with standard forceps can be cultured after placing them in either saline (analysis within 4 h) or transport medium (analysis after up to 24 h) or freezing them at –70 °C or in liquid nitrogen (delayed analysis).

(*a*) *Rapid urease test*

The urease in *H. pylori* breaks down urea into carbon dioxide and ammonia; as ammonia raises the pH, a positive reaction can be read on a pH indicator within a few minutes (Langenberg *et al.*, 1984). Urease tests are agar-based, designed for use in hospital and give results in less than 1 h; their sensitivity has been reported to be 80–98% and their specificity close to 100% (Marshall *et al.*, 1987). Clinical experience indicates, however, that this test may not be specific enough to test the success of treatment. A reading at 24 h increases the sensitivity but decreases the specificity.

(*b*) *Histological examination*

Sections, which must include the superficial and foveolar epithelium, are fixed in formaldehyde or Bouin solution. They can be stained with the standard haematoxylin–eosin stain (Taylor *et al.*, 1987), also used in grading gastritis, but most researchers favour the modified Giemsa stain because better contrast with the background is obtained (Gray *et al.*, 1986). *H. pylori* is best seen under oil immersion. A positive result is expressed semi-quantitatively according to the histological subclassification of the Sydney system (see pp. 207–208) (Price, 1991)

The sensitivity and specificity of histological examination for detecting *H. pylori* depend on the observer's experience. Specificity can be impaired by the presence of other spiral bacteria or coccoidal bacteria, and interpretation may be difficult when only a small number of bacteria are present. Histological methods are best for detecting the non-culturable *Helicobacter*, *H. heilmannii* (Heilmann & Borchard, 1991).

(*c*) *Bacteriological tests*

Smears are prepared by scraping a biopsy specimen with the mucus side against the slide. Gram staining allows observation of curved and spiral gram-negative bacteria. This is a quick, simple and inexpensive test with a sensitivity of about 80% (Montgomery *et al.*, 1987).

Culture is the best means of identifying most infectious agents, because the presence of even one bacterium in the specimen can result in the growth of colonies, allowing precise identification of the organism. For optimal recovery of *H. pylori*, biopsy specimens should be ground, and fresh media containing blood, preferably of human origin, should be used (Westblom *et al.*, 1991). 2,3,5-Triphenyltetrazolium chloride can be included in the medium in order to detect early *H. pylori* colonies (Queiroz *et al.*, 1987). Both selective and non-selective media should be inoculated (Tee *et al.*, 1991), and the culture should be incubated in a microaerobic atmosphere at 37 °C for up to 10 days.

H. pylori colonies are identified by microscopic examination and biochemical tests (see above). Antimicrobial susceptibility tests and molecular fingerprinting can be undertaken in cultures. Since acquired resistance has been noted to four groups of agents used to eradicate *H. pylori*—nitroimidazoles, macrolides, fluoroquinolones and rifamycins, resistance—must be monitored in clinical trials (Glupczynski *et al.*, 1991).

(*d*) *Polymerase chain reaction*

The primers used for detection of *H. pylori* by the polymerase chain reaction (PCR) correspond to genes that encode urease (Labigne-Roussel *et al.*, 1989), 16S ribosomal RNA (Ho *et al.*, 1991), a specific 26-kDa [40-base-pair] protein (Hammar *et al.*, 1992) and an uncharacterized 1.9-kilobase-pair fragment of chromosomal DNA (Valentine *et al.*, 1991). No one pair of primers has proved to be superior to another, but the use of two pairs of primers from different genes may increase specificity. PCR can be used to detect specific genes of pathogenic relevance, such as the *cag*A gene (Figura & Crabtree, 1994).

1.2.2 *Methods based on gastric juice samples*

The techniques used for gastric biopsy specimens can also be used for gastric juice samples. PCR is equally reliable for gastric juice and biopsy specimens (Westblom *et al.*, 1993a). Culture is less sensitive when performed with gastric juice, probably because viable *H. pylori* are lost during prolonged contact with acid (Freland & Drugeon, 1988).

1.2.3 *Methods based on faecal specimens*

Techniques based on faecal specimens are still in an early stage of development. *H. pylori* has been cultured from faeces of infants in the Gambia (Thomas *et al.*, 1992) and has been detected by PCR in faeces (Mapstone *et al.*, 1993), although faecal inhibitors of the reaction remain a problem.

1.2.4 *Methods based on dental plaque and saliva samples*

H. pylori has also been cultured from dental plaque (Krajden *et al.*, 1989) and saliva (Ferguson *et al.*, 1993). Use of PCR has been reported, but these techniques cannot be used as diagnostic methods.

1.2.5 *Methods based on blood samples*

The systemic immune response present in 98% of infected individuals (Glupczynski *et al.*, 1992) can be used for the serological diagnosis of infection (Dooley *et al.*, 1989). Cross-reactions to *C. jejuni* may occur (Newell, 1987). After infection, IgG antibodies are detected within a few weeks. Where it has been validated, the sensitivity and specificity of an enzyme-linked immunosorbent assay (ELISA) with IgG are greater than 90%. Ideally, such tests should be standardized in the population under study; however, it may sometimes be difficult to identify a sufficient number of uninfected people as controls. When *H. pylori* has been eradicated, titres decrease consistently after six months (Kosunen *et al.*, 1992). Immunoblotting allows the detection of a *H. pylori*-specific 120–128-kDa [182–194-base-pair] cytotoxin-associated protein, the *cag*A gene product (Crabtree *et al.*, 1991; Tummuru *et al.*, 1993).

1.2.6 *Urea breath test*

Urea can be hydrolysed by the strong urease of *H. pylori*. In the urea breath test, urea labelled with $^{13}CO_2$ is absorbed and subsequently eliminated in the breath. Breath samples are collected before and 30 min after absorption of labelled urea and analysed by mass spectrometry (Graham *et al.*, 1987). A European protocol has been proposed for this test (Logan *et al.*, 1991). Similar tests involve the use of ^{14}C-urea, as $^{14}CO_2$ can be measured easily with a scintillation counter, but some concern has been expressed over the use of a radioactive isotope. Low-dose tests are being developed to overcome this problem (Bell *et al.*, 1987).

1.3 Epidemiology of infection

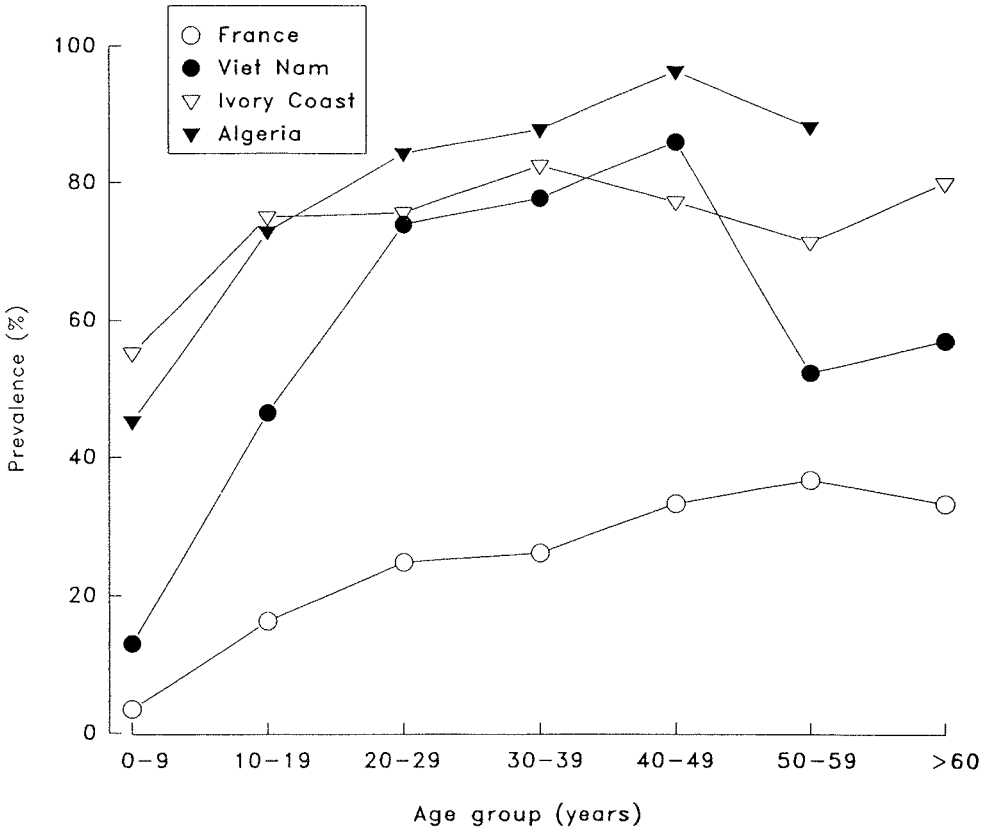
H. pylori infection is long-standing and only rarely resolves spontaneously; it may occasionally be influenced by concomitant antimicrobiological treatment. Thus, it is the prevalence of this infection rather than its incidence that is usually estimated in epidemiological studies (Langenberg *et al.*, 1988; Kuipers *et al.*, 1993a).

1.3.1 *Prevalence*

The prevalence of *H. pylori* infection has been estimated in all the continents on the basis of the results of serological tests on populations such as blood donors, individuals presenting themselves to health centres and volunteers recruited in different ways.

In developing countries, the prevalence of infection increases rapidly during childhood and adulthood and is usually 80–90%. The prevalence is substantially lower in developed countries, especially in childhood (see section 1.3.2). These findings are illustrated in a study in which the same ELISA technique was used in subjects from four countries with different geographical and socioeconomic status (Mégraud *et al.*, 1989) (Figure 1). Similar results were reported in the EuroGast study, in which defined populations of two age groups, 25–34 and 55–64 years, from 17 geographical areas, mainly European, were studied by the same protocol (EuroGast Study Group, 1993a,b; Table 1).

Figure 1. Distribution of seropositivity for *Helicobacter pylori* immunoglobulin G antibodies by age and country of origin



From Mégraud *et al.* (1989)

1.3.2 *Risk factors for infection*

The difference in prevalence between developed and developing countries seems to be linked to socioeconomic factors rather than to ethnicity. In most developed countries, the poorest people also have the highest prevalence. A low level of education and poor housing conditions have been associated with infection (Al Moagel *et al.*, 1990; Fiedorek *et al.*, 1991; Sitas *et al.*, 1991; EuroGast Study Group, 1993a).

No difference in seroprevalence has been found between men and women (Mégraud *et al.*, 1989), and no consistent association has been found with smoking or drinking habits (EuroGast Study Group, 1993a) or any particular diet (Hansson *et al.*, 1993a; Palli *et al.*, 1993). One study in Japan showed a positive association between eating salty food and infection with *H. pylori* (Tsugane *et al.*, 1994). No sexual transmission has been observed (Polish *et al.*, 1991). In cross-sectional studies, an association is always observed between prevalence of infection and age (see Figure 1 and Table 1). Two mechanisms may contribute to this age pattern of prevalence: an age effect, i.e. the progressive acquisition of infection throughout adult life (Graham *et al.*, 1991), and a cohort effect, i.e. a progressive reduction of the rate of infection early in life of people in successive birth cohorts. The extent to which

Table 1. Prevalence of seropositivity to *Helicobacter pylori* in 17 populations

Country	Centre	*H. pylori* seropositivity (%)				
		25–34 years		55–64 years		Total sample
		Male	Female	Male	Female	
Algeria	Algiers	42	44	49	69	200
Belgium	Ghent	20	17	60	47	208
Denmark	Copenhagen	23	5	34	27	157
Germany	Augsburg	14	22	57	65	187
	Deggendorf	40	40	74	76	198
	Mosbach	24	33	65	75	158
Greece	Crete	53	54	80	70	229
Iceland	S. Region	31	40	56	62	206
Italy	Florence	17	14	38	57	205
Japan	Miyagi	55	64	88	87	186
	Yokote	70	54	90	80	200
Poland	Adamowka	69	70	79	93	171
Portugal	Gaia	57	57	73	65	132
Slovenia	Ljubljana	51	27	71	70	201
United Kingdom	Oxford	8	8	49	42	158
	Stoke	27	10	49	41	200
USA	Minneapolis-St Paul	13	16	36	32	198

From EuroGast Study Group (1993b)
NA, not available

these two effects contribute to the cross-sectional association of age with prevalence of infection may vary between populations. The following observations indicate the relative importance of a cohort effect:

- Infection is more strongly correlated to risk factors present during childhood (crowding, size of the family, sharing a bed) than to current risk factors (Mendall *et al.*, 1992; Mitchell *et al.*, 1992; Whitaker *et al.*, 1993; Webb *et al.*, 1994).
- Among adults in developed countries, new cases of infection are uncommon (Mégraud *et al.*, 1989; Rautelin *et al.*, 1990).
- Crude rates of seroconversion from negative to positive have been estimated to be around 0.3–0.5% per year (Parsonnet *et al.*, 1992; Kuipers *et al.*, 1993a); recurrence of infection after eradication therapy may reflect recrudescence of the treated infection rather than true reinfection.
- The cohort effect has been demonstrated in a cohort in the USA (Parsonnet *et al.*, 1992) and in a cohort in the Netherlands (Kuipers *et al.*, 1993a) as well as in a study performed in the United Kingdom. In the last study, sera collected from the same area in 1969, 1979 and 1989, tested for *H. pylori* antibodies by immunoblot and plotted by age group showed that at a given age the prevalence had decreased over the two decades (26% per decade) (Banatvala *et al.*, 1993).

In some populations, a decrease in seroprevalence has been observed in older people. This finding has been attributed to the disappearance of *H. pylori* from the gastric mucosa (loss of *H. pylori* infection) when atrophy develops as a result of long-standing gastritis. Such loss has been observed in some populations (Karnes *et al.*, 1991; Kuipers *et al.*, 1994b) but not in another (Guarner *et al.*, 1993). Furthermore, it is still unclear whether a gradual decrease in *H. pylori* colonization also leads to negative seroconversion. Negative seroconversion was claimed in one retrospective study (Crabtree *et al.*, 1993a) but not in two prospective studies (Parsonnet *et al.*, 1992; Kuipers *et al.*, 1994a).

The prevalence of infection is consistently higher in institutionalized children than in control groups from the surrounding area (Berkowitz & Lee, 1987; Pérez-Pérez *et al.*, 1990).

For a long time, the stomach was thought to be sterile, and precautions such as the use of gloves were not taken in performing endoscopies. A higher prevalence of *H. pylori* infection has now been found among gastroenterologists who perform endoscopies than among other physicians or dentists (Mitchell *et al.*, 1989). In countries with a high prevalence of infection, endoscopists have, nevertheless, a lower prevalence than the general population, probably due to the fact that they come from the middle and upper classes (Matysiak-Budnik *et al.*, 1994).

1.3.3 *Routes of transmission*

Reservoirs of *H. pylori* are the digestive tracts of humans and some primates. Transmission from reservoirs is considered to be person-to-person. This assumption is supported by the finding of clustering of similar strains within families, as shown by molecular fingerprinting (Bamford *et al.*, 1993) and by the consistent demonstration of close interpersonal contact as a risk factor for infection. The *H. pylori* status of mothers of *H. pylori*-positive children is significantly different from that of mothers of *H. pylori*-negative children, indicating that the intimate contact between mother and child could be a cause of transmission (Drumm *et al.*, 1990). Transmission can exist between couples: 68% of spouses of *H. pylori*-infected people were infected, whereas 9% of spouses of uninfected people were infected (Malaty *et al.*, 1991). In another study, the association disappeared in a multiple logistic regression analysis (Pérez-Pérez *et al.*, 1991). Two modes of transmission have been proposed: oral–oral and faecal–oral transmission.

(*a*) *Evidence for faecal–oral transmission*

H. pylori is eliminated in faeces after turnover of the gastric mucosa. It has been detected by PCR (Mapstone *et al.*, 1993) and by culture (Thomas *et al.*, 1992). Consumption of raw vegetables fertilized with human faeces was found to be a risk factor for infection in Santiago, Chile (Hopkins *et al.*, 1993), and consumption of municipal water was found to be a risk factor in children in Lima, Peru (Klein *et al.*, 1991). *H. pylori* has been detected by PCR in sewage water in Peru (Westblom *et al.*, 1993b).

(*b*) *Evidence for oral–oral transmission*

H. pylori has been detected in the oral cavity (Mapstone *et al.*, 1993) and in the saliva of one person (Ferguson *et al.*, 1993). Several claims have been made of the detection of *H. pylori* by PCR in dental plaque (Krajden *et al.*, 1989; Majmudar *et al.*, 1990). When

gnotobiotic puppies infected with *H. felis* were put together with uninfected litter-mates in a germ-free isolator, with continual oral–oral contact, the agent was transmitted. Transmission did not occur between germ-free mice, which are coprophageous, under the same conditions (Lee *et al.*, 1991).

1.4 Clinical disease in humans (other than cancer)

1.4.1 *Gastritis*

H. pylori is a major cause of gastritis. This inference is based on the following observations: (i) ingestion of *H. pylori* led to acute gastritis in a small number of case studies (Marshall *et al.*, 1985a; Morris & Nicholson, 1987; Sobala *et al.*, 1991); (ii) *Helicobacter* colonization of the stomach is virtually always accompanied by inflammation of the mucosa (Dixon, 1991); (iii) *H. pylori* infection can be detected in more than 85% of patients with inflammation of the gastric mucosa (Dooley *et al.*, 1989); and (iv) this inflammation disappears completely within two to three years after eradication of the infection (Rauws *et al.*, 1988; Genta *et al.*, 1993a).

The infection disappears only as a result of antibiotic therapy, after the development of unfavourable gastric conditions such as mucosal atrophy or after partial gastrectomy with bile reflux (Karnes *et al.*, 1991; Kuipers *et al.*, 1993a). 'Spontaneous' clearance of infection is very rare and may in fact be due to unreported use of antibiotics (Kuipers *et al.*, 1993a). In some infected individuals, endoscopic signs of gastritis can be found. The gastritis affects predominantly the antrum (Tytgat *et al.*, 1993), although corpus involvement is observed histologically in most infected individuals (see also section 4).

1.4.2 *Duodenal ulcer disease*

H. pylori infection is the most significant risk factor for duodenal ulcer disease. After exclusion of a small subset of cases of duodenal ulcer with specific etiology, such as use of non-steroidal anti-inflammatory drugs, Crohn's disease or ischaemia, the remaining cases are caused by *H. pylori* (Mégraud & Lamouliatte, 1992). The main arguments for a causal relationship between *H. pylori* infection and duodenal ulcer disease are that the infection is seen to precede the disease (Sipponen *et al.*, 1990) and that the disease disappears after treatment of the infection. While ulcers have been shown in many studies to relapse within 12 months after symptomatic treatment in 50–100% of patients (Tytgat *et al.*, 1993), eradication of *H. pylori* almost totally prevents ulcer recurrence (Marshall *et al.*, 1988; Graham *et al.*, 1992; Tytgat *et al.*, 1993). It has been estimated that up to 10% of infected people will develop duodenal ulcer during life (Tytgat *et al.*, 1993).

1.4.3 *Gastric ulcer disease*

H. pylori infection is present in approximately 70% of patients with gastric ulcers (Labenz & Börsch, 1994). A variety of noxious agents such as non-steroidal anti-inflammatory drugs and bile reflux are risk factors for the development of gastric ulcers. After exclusion of patients with those risk factors, the bacterium is present in more than 95% of the remaining cases. Eradication of the infection significantly prevents ulcer recurrence (Graham *et al.*, 1992; Labenz & Börsch, 1994).

1.4.4 *Hypertrophic protein-losing gastritis*

Hypertrophic protein-losing gastritis is a rare clinical disorder characterized by chronic gastritis with giant folds, gastric protein loss and hypoalbuminaemia. The etiology of this disorder is unknown. Significant clinical improvement was seen after *H. pylori* eradication therapy in two studies (Lepore *et al.*, 1988; Meuwissen *et al.*, 1992).

1.4.5 *Childhood diseases*

In children in developing countries, *H. pylori* infection has been associated with chronic diarrhoea and malnutrition (Sullivan *et al.*, 1990). In developed countries, it has also been associated with chronic abdominal pain and growth retardation.

1.5 Treatment and control

1.5.1 *Antibiotics and acid suppressive therapy*

Since the introduction of H2-blockers and proton pump inhibitors, *H. pylori*-related disorders have been treated with moderate success (Susi *et al.*, 1994). The effects of acid suppressive medication on *H. pylori*-related gastritis have not been examined adequately; however, such medication does not cure the infection (Kuipers *et al.*, 1993b). The bacterium is sensitive to a wide range of antibiotics *in vitro*, but most are unsuccessful *in vivo*. Three strategies have been chosen to overcome this problem: (i) combination of multiple synergistic antibiotic drugs; (ii) prolongation of drug administration; and (iii) combination of antibiotics with acid suppressors. A large number of clinical trials have been carried out to find an effective treatment regimen. The current preference is for therapy lasting 14 days with either two antibiotics combined with a bismuth preparation or with one to two antibiotics combined with an acid inhibitor, usually omeprazole (Labenz *et al.*, 1993). With these regimens, eradication has been achieved in 60–95% of cases, depending upon the prevalence of antibiotic-resistant strains and patient compliance.

1.5.2 *Vaccination*

H. pylori infection is always accompanied by local and systemic immune responses, with no clearance of infection (Bontkes *et al.*, 1992). It is thus unclear whether immunization can prevent new infections. Successful oral immunization of mice with a sonicated preparation of *H. felis* plus adjuvant (cholera toxin) has been achieved (Chen *et al.*, 1993).

2. Studies of Cancer in Humans

2.1 Descriptive studies

2.1.1 *Geographical correlations*

(*a*) *Gastric carcinoma*

Table 2 lists eight studies in which the prevalences of *H. pylori* infection were compared in geographical regions with different gastric cancer rates. The presence of infection was

Table 2. Geographical correlation studies of the prevalence of *Helicobacter pylori* infection and incidence or mortality rates for gastric cancer

Country	Populations	Total number of people surveyed	Gastric cancer		*H. pylori* infection		Results of comparison	Reference
			Period	Range of occurrence (rate)	Period	Range of prevalence (%)		
Colombia	Gastrointestinal patients, aged 15–84; 1 low-risk, 1 high-risk city	78	1972–81	Incidence, 26–150/100 000	NR	63–93	$p = 0.01$	Correa *et al.* (1990a)
Costa Rica	Healthy individuals, aged 7–20; 1 low-risk, 1 high-risk rural area	282	1984–88	Incidence, 20–49/100 000	NR	66–72	$p > 0.05$	Sierra *et al.* (1992)
Italy	Population sample, aged 35–74; 3 high-risk, 2 low-risk areas	930	1975–77	Mortality, 3–43/100 000	1985–88	44–45	$p > 0.05$	Buiatti *et al.* (1989a); Palli *et al.* (1993)
China	Gastrointestinal patients, aged 17–72; 1 low-risk, 1 medium-risk, 1 high-risk area	690	1985–87	CM, 8–60/ 100 000	NR	13–63[a]	$p < 0.01$	Lin *et al.* (1989)
Japan	Blood donors, aged 16–64; 4 prefectures	1815	1982–87	SM, 48–136 (M), 40–117 (F)	NR	50–60 (M) 41–60 (F)	[$r = 0.01$, $p > 0.05$ (M) $r = -0.57$, $p > 0.05$ (F)]	Fukao *et al.* (1993)
Japan	Population sample, aged 40–49, men, 5 areas	624	1985–89	CM, 0–74, 2.2–5.7%	NR	63–86	$r = 0.75$ [$p = 0.14$]	Tsugane *et al.* (1993)
China	Population sample, men aged 35–64; 46 rural counties	1882	1973–75	CM, 0–64, 0.3–6.9%	1983	28–96	$r = 0.34$[c] [$p = 0.02$]	Forman *et al.* (1990)
13 countries	Population sample, aged 25–34 and 55–64; 17 areas or cities (16 with data on mortality, 11 with data on incidence)	3194	Early–mid-1980s	CI, 0–74, 0.9–9.9% (M) 0.3–4.0% (F) CM, 0–74, 0.6–5.7% (M) 0.2–2.1% (F)	NR	8–70 (25–34 years) 31–87 (55–64 years)	$\beta = 1.79$ ($p = 0.002$) (M) $\beta = 2.68$ ($p = 0.001$) (I)	EuroGast Study Group (1993b)

NR, not reported; CM, cumulative mortality; SM, standardized mortality; CI, cumulative incidence; (M), males; (F), females

[a]Based on gastric biopsy

[b]Other cancer sites also studied

[c]$r = 0.40$ after adjustment for within-county variability

determined in most studies by ELISA for IgG antibodies to *H. pylori* in serum. In all studies, infection rates were compared with cancer rates in contemporaneous time periods, although a more appropriate comparison would be between infection prevalence rates and cancer rates several years or even decades later. Such a comparison would reflect the time sequence involved if there were a causal relationship between infection and cancer.

Four of the studies were comparisons of regions of high and low risk for gastric cancer within a single country; two showed a significant difference between *H. pylori* prevalence rates, with an increase in the high-risk region (in Colombia, Correa *et al.*, 1990a; and in China, Lin *et al.*, 1989), while the other two showed no significant difference between the two regions (in Italy, Palli *et al.*, 1993; and in Costa Rica, Sierra *et al.*, 1992). Two studies from Japan (Fukao *et al.*, 1993; Tsugane *et al.*, 1993) compared populations within five and four areas, respectively; neither showed a significant association between *H. pylori* seropositivity and gastric cancer mortality.

Forman *et al.* (1990) examined the prevalence of *H. pylori* IgG antibodies in 1882 residents of 46 rural counties in China and compared them with the gastric cancer mortality rates in the same counties. The correlation between *H. pylori* antibody prevalence rate and gastric cancer mortality rate was 0.34 ($p = 0.02$). The significant positive correlation remained after adjustment for dietary factors associated with risk for gastric cancer (Kneller *et al.*, 1992).

The EuroGast Study Group (1993b) examined the seroprevalence of *H. pylori* IgG antibodies in 3194 randomly selected subjects resident in 17 centres in 13 countries, chosen to reflect the global range in gastric cancer incidence. In regression analyses, in which the two sexes were combined, there were significant relationships between the prevalence of *H. pylori* antibodies and both log-transformed gastric cancer cumulative mortality ($p = 0.002$) and incidence ($p = 0.001$) rates. Exclusion of the regions with highest and lowest mortality rates (Japan and the USA, respectively) reduced the strength of the relationship with mortality from gastric cancer to a nonsignificant ($\beta = 0.62; p = 0.3$) level (Forman *et al.*, 1993).

It has been noted (Holcombe, 1992) in Nigeria and other African countries (e.g. Sudan, Uganda and Zimbabwe) that gastric cancer rates are relatively low (< 2–3% of all malignant tumours) despite a very high prevalence of *H. pylori* infection. The populations of other developing countries with low incidence rates of gastric cancer, but for which no estimates of the prevalence of infection are available, include Kuwaitis, non-Jews in Israel, Malays in Singapore and those of Ahmedabad, Bangalore, Madras and Bombay in India. Gastric cancer incidence rates in the three population-based cancer registries in Africa (Sétif, Algeria; Bamako, Mali; and the Gambia) range from 3.9 to 19.4 per 100 000 in males and from 1.5 to 10.3 per 100 000 in females (Parkin *et al.*, 1992). These rates are substantially below those in high-risk regions of the world (e.g. Costa Rica: 46.9 in males and 21.3 in females) and are comparable to the rates in US blacks (12.4 in males and 5.6 in females) and in England and Wales (16.9 in males and 6.8 in females).

(*b*) *Gastric lymphoma*

Doglioni *et al.* (1992) compared the incidence of primary gastric lymphoma, determined from endoscopy clinic records, in an area of northeastern Italy with that in three communities

in the United Kingdom. In the Italian city of Feltre, the estimated incidence rate for gastric lymphomas was 66/100 000 per five years for the period 1986–90 (37 cases). In three districts in the United Kingdom, the comparable rates were 6/100 000 (six cases), 4/100 000 (seven cases) and 6/100 000 (20 cases). The *H. pylori* infection rate of all patients undergoing endoscopic biopsy was 87% in Feltre in 1991 and 50–60% in the United Kingdom. [The Working Group noted that this was a hospital-based study with no information about the referral patterns to the local endoscopy units. There is, therefore, uncertainty about the denominator populations used in this study.]

(c) *Other cancers*

In the study of Forman *et al.* (1990) (see above), correlation coefficients were calculated for associations between *H. pylori* IgG antibody prevalence and mortality rates from cancers at 12 sites other than the stomach. None was significant. The correlation with lymphoma (all types) was 0.32 and of borderline significance.

2.1.2 *Time trends*

Gastric cancer incidence and mortality rates have been declining rapidly in nearly all developed countries for several years. There are few data for developing countries, but the same trend has generally been observed (Coleman *et al.*, 1993). Secular trends in the prevalence of *H. pylori* infection have not been investigated extensively, but the one serological study that has been conducted in the United Kingdom (Banatvala *et al.*, 1993) indicated that the prevalence has decreased in recent decades. If *H. pylori* is acquired predominantly in childhood (see section 1.3.2), then data on age prevalence (section 1.3.1) can be interpreted as indicating a declining prevalence rate over much of the 20th century. This is also consistent with observed secular trends in duodenal ulcer disease in the USA (Sonnenberg, 1993), the United Kingdom (Susser & Stein, 1962) and Europe (La Vecchia *et al.*, 1993), a disease strongly associated with *H. pylori* infection. Data from Japan (Blaser, 1993) indicate that mortality from gastric cancer in that country has decreased over the past 50–80 years, an effect consistent with a secular decrease in exposure to an environmental agent. The prevalence of gastric cancer of the cardia, in contrast to that of more distal sites within the stomach, has been shown to be increasing in a number of populations (Powell & McConkey, 1990; Blot *et al.*, 1991; Hansson *et al.*, 1993b). Gastric cancer of the cardia has been shown in some studies (Talley *et al.*, 1991a; Hansson *et al.*, 1993b) not to be associated with *H. pylori* infection (see sections 2.3 and 2.4).

2.1.3 *Socioeconomic trends*

Gastric cancer has been shown consistently in several countries to be commoner in poorer socioeconomic groups (Howson *et al.*, 1986; Buiatti *et al.*, 1989b; Logan, 1982). The same association has been observed consistently for *H. pylori* infection (see section 1.3.2).

2.2 Case series

2.2.1 *Gastric carcinoma*

The presence of *H. pylori* infection has been determined in numerous series of gastric cancer patients, usually by histological examination of biopsy and/or gastrectomy samples

but also by microbiological culture; in some studies, serological tests were used to determine the presence of specific IgG antibodies to *H. pylori*. A number of studies were designed specifically to estimate the prevalence of *H. pylori* infection in gastric cancer patients; the majority, however, were broader surveys of patients with upper gastrointestinal disease and included a small subgroup of patients with gastric cancer. In the latter studies, it is unclear whether adequate mucosa was available to evaluate the presence of *H. pylori*; there was also frequently a subgroup of patients who had dyspeptic symptoms but no lesions in their stomachs and who were used as a control series. In a few studies, the control series were healthy volunteers who had undergone endoscopy. Serologically based studies in which data from matched case and control series were available are summarized in sections 2.3 and 2.4.

Table 3 lists the 11 largest case series. The percentage of gastric cancer patients who had *H. pylori* infection varied from 43 to 83%. Particular interest has focused on the Laurén histological classification of gastric adenocarcinoma into cancers of the intestinal (glandular) type and cancers of the diffuse type (Laurén, 1965). It has been reported that the incidence of the former varies between populations whereas that of the latter remains relatively constant (Laurén, 1965; Muñoz *et al.*, 1968; Muñoz & Asvall, 1971; Correa *et al.*, 1973). Environmental exposures are thought to be more important in the etiology of intestinal-type than of diffuse-type cancers (Howson *et al.*, 1986). Table 4 lists eight series in which the cancer cases were classified into intestinal and diffuse histological categories. In some of these studies, an increased prevalence of *H. pylori* infection was seen in association with intestinal-type cancers (Parsonnet *et al.*, 1991a; Tatsuta *et al.*, 1993), but this difference was not observed consistently.

2.2.2 *Gastric lymphoma*

Wotherspoon *et al.* (1991) examined 110 patients in the United Kingdom with gastric B-cell mucosa-associated lymphoid tissue lymphomas, a subset of primary gastric lymphomas. In this group, 101/110 patients (92%) had histological evidence of *H. pylori* infection.

A total of 205 surgical specimens containing primary malignant B-cell lymphomas were investigated in Germany. *H. pylori* colonization was found in 175/178 (98%) cases in which the mucosa some distance from the tumour could be evaluated (Stolte *et al.*, 1994).

2.3 Cohort studies

2.3.1 *Gastric carcinoma*

Four prospective studies have been reported in which the relationship between *H. pylori* infection and the subsequent risk of gastric cancer has been assessed. All were case–control comparisons nested in prospective cohort studies in which blood samples had been taken from cancer-free individuals and stored. Specific antibodies to *H. pylori* were then measured in blood samples from individuals who subsequently developed gastric cancer, and the proportion of individuals with antibodies was compared with that in a matched control group.

Table 3. Prevalence of *Helicobacter pylori* in series of gastric cancer cases

Country	Gastric cancer cases			Method of assessment	Comments	Reference
	No.	*H. pylori* infection				
		No.	%			
Europe						
Italy	44	26	59	Histology	22/44 cases were 'early' gastric cancer, 17 (77%) positive	Caruso & Fucci (1990) (letter)
Italy	277	216	78	Histology	137/167 (82%) early gastric cancers positive; 79/110 (72%) advanced gastric cancers positive	Fiocca *et al.* (1993)
Netherlands	91	54	59	Histology		Loffeld *et al.* (1990)
Turkey	46	34	74	Histology		Buruk *et al.* (1993)
United Kingdom	136	67	49	Histology		Armstrong *et al.* (1991) (letter)
United Kingdom	224	96	43	Histology		Clarkson & West (1993)
North America						
USA	59	40	68	Histology		Parsonnet *et al.* (1991a)
South America						
Brazil	40	33	83	Histology	18/19 (94%) cases positive by histology and culture	Nogueira *et al.* (1993)
Asia						
Japan	94	66	70	Serology		Takahashi *et al.* (1993)
Japan	41	24	59	Culture	All tumours were 'early' gastric cancers	Tatsuta *et al.* (1993)
Singapore	137	103	75	Histology		Wee *et al.* (1992)

Table 4. Prevalence of *Helicobacter pylori* infection in gastric cancer case series by histological type (Laurén classification)

Country	Histological classification[a]						Reference
	Intestinal			Diffuse			
	Total no.	*H. pylori* infection		Total no.	*H. pylori* infection		
		No.	%		No.	%	
Europe							
Italy	166	119	72	79	71	90	Fiocca *et al.* (1993)
Netherlands	80	48	60	11	5	45	Loffeld *et al.* (1990)
Turkey	26	23	88	20	11	55	Buruk *et al.* (1993)
United Kingdom	120	56	47	69	24	35	Clarkson & West (1993)
North America							
USA	37	33	89	22	7	32	Parsonnet *et al.* (1991a)
South America							
Brazil	31	24	77	5	5	100	Nogueira *et al.* (1993)
Asia							
Japan[b]	24	19	79	17	5	29	Tatsuta *et al.* (1993)
Singapore	87	64	74	50	39	78	Wee *et al.* (1992)

[a]Method of assessment shown in Table 3.
[b]In this study the terms 'differentiated early gastric cancer' and 'undifferentiated early gastric cancer' were used for intestinal and diffuse, respectively.

Forman *et al.* (1991) compared 29 gastric cancer patients with 116 age-matched controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Steer *et al.*, 1987) with a reported sensitivity of 93% and a specificity of 96% (Talley *et al.*, 1991b). The subjects were all men taking part in one of two cohort studies: in one study, 20 179 men, aged 35–64 and living in south-east England, provided blood between 1975 and 1982 during a health check-up. In the other study, 2512 men, aged 45–59 and living in Caerphilly, Wales, provided blood between 1979 and 1982 as part of a population study of cardiovascular disease. Cancers or deaths among cohort participants were notified to the study organizers routinely; 23 men with gastric cancer were identified from the first cohort and six from the second. Cancers were diagnosed between 1980 and 1989, with a mean interval between blood sampling and diagnosis of six years (range, four months to 13 years seven months). The mean age of the cancer patients was 54 years (range, 41–63 years) at blood sampling and 60 years (range, 47–76 years) at diagnosis. Four controls were selected for each case by

matching on cohort, date of birth (within one year), date of blood sampling (within one year) and number of freeze–thaw cycles the blood sample had undergone. Twenty of the 29 (69%) gastric cancer patients and 54 of the 116 (47%) controls had antibodies to *H. pylori*, resulting in a matched odds ratio of 2.8 (95% confidence interval [CI], 1.0–8.0). Stratifying the cases and corresponding controls into those diagnosed within five years of blood sampling and those diagnosed five or more years after sampling did not result in a significant difference in the resulting odds ratios. No information was available on site of cancer within the stomach or on histological subtype.

Parsonnet *et al.* (1991b) compared 109 gastric patients with 109 age-, sex- and race-matched controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Evans *et al.*, 1989) with a reported sensitivity of 91% and a specificity of 98%. The subjects were taking part in a cohort study in which 128 992 participants living in California, USA, provided blood between 1964 and 1969 during a health check-up. A total of 246 gastric cancer registrations and/or hospitalizations for gastric cancer among cohort participants were notified to the study organizers routinely, and 200 of these were randomly selected. Availability of blood samples resulted in final inclusion of 186 patients with gastric cancer. Cancers were diagnosed between 1964 and 1989, with a mean interval between blood sampling and diagnosis of 14.2 years (range, 1–24 years). One control was selected for each case by matching on age at blood sampling (within one year), sex, race, date of blood sampling (within 0.5 year) and site of the health check-up. Of the 186 patients, 109 had histologically confirmed adenocarcinoma of the stomach; of these, 92 (84%) had antibodies to *H. pylori*, as did 66 of the 109 (61%) controls, resulting in a matched odds ratio of 3.6 (95% CI, 1.8–7.3). When the cases and controls were stratified by sex, the odds ratio for women was nonsignificantly higher than that for men; when they were stratified by race, the odds ratio for blacks was nonsignificantly higher than that for whites. Eighty-one patients had the intestinal type of adenocarcinoma (Laurén classification), and 67 (83%) of these were seropositive (odds ratio, 3.1; 95% CI, 1.5–6.6); 28 patients had a diffuse type, and 25 (89%) of these were seropositive (odds ratio, 8.0; 95% CI, 1.0–64). Four patients had an adenocarcinoma at a site in the cardia; one was seropositive, as was one of the four matched controls. An additional 27 patients had adenocarcinoma of the gastroesophageal junction (not included in the main analyses above); of these, 17 (63%) were seropositive, as were 19 (70%) controls (odds ratio, 0.8; 95% CI, 0.3–2.1).

Nomura *et al.* (1991) compared 109 patients with gastric carcinoma with 109 age-matched controls for the presence of IgG antibodies to *H. pylori* using a commercial ELISA. The subjects were all men taking part in a cohort study in which 7498 Japanese–Americans living in Oahu, Hawaii, USA, provided blood between 1967 and 1970 as part of a population study of heart disease. A total of 137 gastric cancer registrations and/or hospital discharges for gastric cancer among cohort participants were notified to the study organizers routinely, all with histologically confirmed gastric cancer. As insufficient serum was available from 26 men and the results of the ELISA were indeterminate for two, a total of 109 were included in the study. Cancers were diagnosed between 1968 and 1989, with a mean interval between blood sampling and diagnosis of 13 years (standard deviation, five years). The mean age of the cancer patients at recruitment was 59 years. One control was selected for each case by matching for age at recruitment and date of blood collection. Excluded from the control

series were men who had had a gastrectomy before blood sampling or who had had a diagnosis of peptic ulcer at any time. The exclusion criteria reduced the pool of available controls by 13%. [The Working Group noted that the exclusion criteria would be likely to reduce the prevalence of *H. pylori* infection in the control group and, hence, bias the estimated odds ratio upwards.] Also excluded were men with cardiovascular disease or any other type of cancer diagnosed at any time. These exclusions reduced the control pool by 33%. Controls had to be alive when the cancer cases with which they were matched were diagnosed. Of the 109 gastric cancer patients, 103 (94%) had antibodies to *H. pylori*, as did 83 of the 109 (76%) controls, resulting in a matched odds ratio of 6.0 (95% CI, 2.1–17). Stratification of the cases into three groups (26, 40 and 43 pairs) on the basis of time between blood sampling and cancer diagnosis resulted in odds ratios of 1.5 (95% CI, 0.3–9.0) for less than 10 years, 6.0 (1.3–27) for 10–14 years and indeterminate (1.7–97) for 15 years or more. Stratification into two birth cohorts resulted in odds ratios of 3.0 (0.8–11) for those born in 1900–09 and 15 (2.0–114) for those born in 1910–19. Eighty-one patients had an intestinal type of carcinoma, and 75 (93%) of these were seropositive (odds ratio, 4.5; 95% CI, 1.5–13); 23 patients had a diffuse type, and all were seropositive (odds ratio, indeterminate; 1.1–64). Five patients had cancer at the cardia, and two were seropositive; after exclusion of these patients, the overall odds ratio was 12 (95% CI, 2.8–51). In this study, a trend was observed ($p = 0.0009$) of an increasing odds ratio with an increase in the quantitative antibody level. [The Working Group estimated that, had the exclusion criteria relating to controls with a history of gastrectomy or peptic ulcer not been used, the prevalence of *H. pylori* infection in the controls would have been increased by approximately 4% and the overall odds ratio would have been decreased by about 20%, i.e. from 6.0 to 4.8.]

In a combined analysis of the three nested case–control studies described above, Forman *et al.* (1994) showed that, overall, 215/247 (87%) gastric cancer patients and 203/334 (61%) controls were seropositive for IgG antibodies to *H. pylori*, resulting in a matched odds ratio of 3.8 (95% CI, 2.3–6.2). When these results were stratified by time between sample collection and cancer diagnosis into four periods—fewer than five years, 5–9 years, 10–14 years and 15 years or more—there was a significant trend ($p = 0.049$) towards an increased odds ratio with increasing time interval. The odds ratio changed from 2.1 (95% CI, 0.6–8.7) to 2.3 (0.9–6.5), 4.4 (1.8–13) and 8.7 (2.7–45) over the four periods, respectively. There were 20/25 (80%), 37/46 (80%), 70/78 (90%) and 88/98 (90%) seropositive cases and 34/58 (59%), 46/85 (54%), 58/93 (62%) and 65/98 (66%) seropositive controls in the four strata, respectively. This trend was interpreted by the authors as indicating that false-negative assessments of *H. pylori* status may have occurred more frequently among cancer cases than among matched controls, especially among those diagnosed soon after providing blood. False-negative assessments were believed to derive from the precancerous conditions, severe atrophic gastritis and intestinal metaplasia, from loss of *H. pylori* colonization and loss of seropositivity.

Lin *et al.* (1993a [abstract]) compared 29 gastric cancer patients in Taiwan, China, with 220 controls matched by age, sex and area of residence, for the presence of *H. pylori* IgG antibodies by an ELISA. The subjects were participants in a cohort study in which 9777 people in Taiwan had provided blood since 1984. The mean interval between blood sampling and diagnosis of cancer was 3.1 years. Sixty-nine percent of the gastric cancer patients and

59% of the controls were seropositive for antibodies to *H. pylori*, resulting in an odds ratio of 1.6 (95% CI, 0.68–2.6).

The four prospective studies are summarized in Table 5.

2.3.2 *Gastric lymphoma*

Parsonnet *et al.* (1994) compared 33 patients with gastric non-Hodgkin's lymphoma with 134 age- and sex-matched controls for the presence of *H. pylori* IgG antibodies using an ELISA with a reported sensitivity of 96% and a specificity of 76% for active gastric infection. The subjects were taking part in one of two cohort studies, one in California, USA, described above (Parsonnet *et al.*, 1991b), and the other of 170 000 participants living in Norway who provided blood between 1973 and 1991 during blood donation and health screening programmes. Cancer registrations between 1973 and 1990 among the Norwegian cohort were notified to the study organizers. Twenty gastric lymphomas were identified from the US cohort and 13 from the Norwegian cohort, with median intervals between blood sampling and diagnosis of 14 and 13 years, respectively. The median ages of the lymphoma patients at diagnosis were 66 and 55 years, and 40 and 69% patients in the two cohorts were men, respectively. Four cancer-free controls were selected for each case and matched on cohort, date of birth, age (five-year groups in the USA; within six months in Norway), sex, date and location of blood collection and ethnic group (only in the USA). Twenty-eight of the 33 (85%) gastric lymphoma patients were seropositive for antibodies to *H. pylori*, as were 74 of the 134 (55%) controls, resulting in a matched odds ratio of 6.3 (95% CI, 2.0–20). There was no significant difference between odds ratios when the cases and corresponding controls were stratified on the basis of cohort, sex, age at diagnosis (< 65 or ≥ 65 years) or time between blood sampling and diagnosis (< 14 or ≥ 14 years). In a separate analysis of 31 patients with non-gastric non-Hodgkin's lymphoma and 61 matched controls, 20 patients (65%) and 36 controls (59%) were seropositive, resulting in a matched odds ratio of 1.2 (95% CI, 0.5–3.0).

2.4 Case–control studies

2.4.1 *Gastric carcinoma*

Nine case–control studies have been carried out in which serological assessment of infection was done retrospectively in cancer patients after diagnosis.

Talley *et al.* (1991a) compared 69 patients with gastric adenocarcinoma with 252 controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Pérez-Pérez *et al.*, 1988) with a reported sensitivity of 96% and a specificity of 94% (Talley *et al.*, 1991b). The cases of cancer had been confirmed histologically and diagnosed between 1982 and 1989 at a single hospital in Minnesota, USA. The median age of the patients was 63 years (25th and 75th percentiles, 56.5 and 71 years), and 52% were men. The controls comprised 76 asymptomatic volunteers with no history of gastrointestinal disease and 176 patients who were treated between 1976 and 1989 at the same hospital as the cancer patients for a variety of non-malignant conditions: 67 for benign musculoskeletal problems, 52 for benign oesophageal disease and 57 for benign lung diseases. The median age of the controls

Table 5. *Helicobacter pylori* seroprevalence rates in gastric cancer patients and matched controls: prospective studies

Country	Cohort	Cases			Controls				Odds ratio[a]	95% CI	Mean follow-up (years)	Reference
		No.	*H. pylori* infection		No.	Matching	*H. pylori* infection					
			No.	%			No.	%				
United Kingdom	English undergoing health check-up; Welsh heart disease study Men	29	20	69	116	Cohort, date of birth, date of blood sampling, no. of freeze–thaw cycles	54	47	2.8	1.0–8.0	6	Forman *et al.* (1991)
USA (California)	Men and women undergoing health check-up	109	92	84	109	Age, sex, rate, date of blood sampling, place of health check-up	66	61	3.6	1.8–7.3	14	Parsonnet *et al.* (1991b)
USA (Hawaii)	Japanese–Americans; heart disease study Men	109	103	94	109	Age, date of blood sampling	83	76	6.0	2.1–17	13	Nomura *et al.* (1991)
China (Taiwan)	General population Men and women	29	20	69	220	Age, sex, residence	130	59	1.6	0.68–2.6	3	Lin et al. (1993a) [abstract]

CI, confidence interval
[a]From matched analysis

was 61 years (25th and 75th percentiles, 54 and 67 years), and 50% were men. Of the 69 gastric cancer patients, 36 (52%) had antibodies to *H. pylori*, as did 96 (38%) of the controls. The odds ratio, after adjustment for age and sex, was 1.6 (99% CI, 0.79–3.4). Adjustment for length of storage of the blood samples had no substantial effect on the results. The odds ratio for gastric cancers at sites other than the cardia ($n = 37$) was 2.7 (99% CI, 1.0–7.1), while that for cancers at sites in the cardia ($n = 32$) was 0.94 (99% CI, 0.34–2.6). For the intestinal type of gastric cancer, according to the Lauren classification ($n = 32$), the odds ratio was 1.9 (99% CI, 0.67–5.1), while for cancers of the diffuse histological type ($n = 22$) it was 2.5 (99% CI, 0.73–8.2). After the cancers of the cardia had been excluded, the odds ratios were 4.6 (99% CI, 0.78–27) for the intestinal type ($n = 13$) and 2.3 (99% CI, 0.63–8.1) for the diffuse type ($n = 19$). There were five additional groups of patients in this study. The proportions with antibodies to *H. pylori* were 44% of nine with benign gastric lesions, 89% of nine with gastric cancers other than adenocarcinoma, 51% of 80 with colorectal cancer, 49% of 41 with oesophageal cancer and 56% of 79 with lung cancer. In comparisons with the cancer-free control group, as used in the study of gastric adenocarcinoma, the odds ratios, after adjustment for age and sex, were 1.5 (99% CI, 0.23–9.1) for benign gastric neoplasms, 13 (99% CI, 0.77–203) for other gastric cancers, 1.8 (99% CI, 0.86–3.4) for colorectal cancer, 1.4 (99% CI, 0.58–3.4) for oesophageal cancer and 1.8 (99% CI, 0.91–3.6) for lung cancer.

Sipponen *et al.* (1992) compared 54 patients with gastric adenocarcinoma with 84 controls for the presence of IgG, IgA and IgM antibodies to *H. pylori* using a previously described ELISA (Kosunen *et al.*, 1989). The cases of gastric cancer were confirmed histologically and occurred in a consecutive series of patients diagnosed in 1988 and 1989 at a single hospital in Finland. Patients with cancers of the region of the cardia were excluded, as were patients who had previously undergone gastric surgery. The mean age of the patients who were included was 65 years (SD, 16 years), and 48% were men. The controls were 35 patients with cancers at gastrointestinal sites other than the stomach (6 in the oesophagus, 7 in the pancreas and 22 in the colon) and 48 patients with cancers at sites other than the gastrointestinal tract. The mean ages of these two groups of controls were 65 years (SD, 12 years) and 66 years (SD, 12 years), respectively, and 57 and 71%, respectively, were men. IgG antibodies to *H. pylori* were found in 38/54 (70%) of the gastric cancer patients and 43/84 (51%) of the patients with other cancers. [The unadjusted odds ratio was calculated by the Working Group to be 2.3 (95% CI, 1.0–5.0).] IgA antibodies to *H. pylori* were found in 76% of the gastric cancer patients and 58% of the controls [the unadjusted odds ratio was 2.3 (95% CI, 1.1–4.8]; IgM antibodies were found in 6% of the cases and 5% of the controls. When the gastric cancer patients were stratified into three age groups, IgG antibodies were found in 8/10 (80%) aged 30–49 years, 13/19 (68%) of those aged 50–69 years and 17/25 (68%) of those aged 70 years or more. For the patients with other cancers, the respective proportions were 5/9 (56%), 22/38 (58%) and 16/37 (43%), resulting in odds ratios for the three strata of [3.2 (95% CI, 0.3–45.4)], [1.6 (0.4–6.2)] and [2.8 (0.9–9.4)], respectively. Thirty-one gastric cancer patients had tumours of the intestinal type, and 22 (71%) of them were seropositive; 21 gastric cancer patients had tumours of the diffuse type, and 15 (71%) of them were seropositive.

Kang and Chung (1992) compared 28 patients with gastric adenocarcinoma in the Republic of Korea with 30 age- and sex-matched controls for the presence of IgG antibodies

to *H. pylori*, using a commercial ELISA kit. The gastric cancer patients had all undergone resection, had histological confirmation of their disease and had been diagnosed in 1991. The mean age of the cases was 50 years (range, 29–67 years), and 66% were men. Controls were hospital patients with a variety of diagnoses other than gastrointestinal disease and included nine patients with non-gastrointestinal cancer. The mean age of the controls was 52 years (range, 28–69 years), and 67% were men. Twenty-five (89%) of the gastric cancer patients had antibodies to *H. pylori*, as did 20 (67%) of the control patients. A matched analysis resulted in an odds ratio of 4.2 (95% CI, 1.0–17). Ten of the patients had intestinal-type cancers, and eight (80%) of these were seropositive; 18 patients had diffuse-type cancers, and 17 (94%) of these were seropositive. All nine gastric cancer patients who had 'early gastric cancer' were seropositive; of the 19 who had advanced cancer, 16 (84%) were seropositive.

Hansson *et al.* (1993a) compared 112 gastric adenocarcinoma patients with 103 controls for the presence of IgG antibodies to *H. pylori* using a commercial ELISA kit with a reported sensitivity of 98.7% and a specificity of 100% (Evans *et al.*, 1989). The cases were confirmed histologically and occurred in a consecutive series of patients diagnosed between 1989 and 1991 at eight hospitals in central and northern Sweden. Patients over 79 years of age and with advanced disease (20% of study base) were excluded, as were patients who refused (3%) or were unable (14%) to give blood. The mean age of the gastric cancer patients was 67 years, and 63% were men. Controls were patients admitted to the same hospitals with a variety of non-gastrointestinal diseases, who were frequency matched to the cases by 10-year age group, sex and hospital. The mean age of the controls was 67 years, and 66% were men. Antibodies to *H. pylori* were found in 90/112 (80%) of the gastric cancer patients and 63/103 (61%) of the controls (odds ratio, 2.6; 95% CI, 1.4–5.0). When the analysis was stratified into three age groups, the odds ratios were 9.3 (1.4–101) for patients aged less than 60 years, 4.3 (1.3–15) for those 60–69 years and 1.2 (0.44–3.0) for those aged 70 or more. The interaction between age and *H. pylori* seropositivity was significant. There was a higher odds ratio in men than in women, but the effect was of borderline significance. The multivariate odds ratio for *H. pylori* seropositivity, estimated in a multiple regression model with adjustment for occupation, diet, smoking and alcohol consumption (multivariate odds ratio, 2.7; 95% CI, 1.3–5.8) showed little difference from the univariate odds ratio. Of patients with gastric cancers at sites other than the cardia, 77/93 (83%) were seropositive (odds ratio, 3.1; 1.5–6.3), while 13/19 (68%) patients with cancers of the cardia were seropositive (1.4; 0.44-4.8). Of patients with intestinal-type gastric cancer, 60/75 (80%) were seropositive (2.5; 1.2–5.4), while 22/28 (79%) of patients with diffuse-type cancer were seropositive (2.3; 0.82–7.6).

Blaser *et al.* (1993) compared 29 gastric adenocarcinoma patients with 58 age- (within one year) and sex-matched controls for the presence of IgG antibodies to *H. pylori*, using a previously described ELISA (Pérez-Pérez *et al.*, 1988) with a reported sensitivity of 96% and a specificity of 94% (Talley *et al.*, 1991b). The cases were confirmed histologically and had been diagnosed between 1990 and 1992 in one city, Ichikawa, in Japan. The median age of patients was 63 years (range, 46–82 years), and 62% were men. Controls were out-patients attending the same hospital as the gastric cancer patients for a variety of illnesses, excluding 'known stomach disease' and chronic liver disease. Twenty-four of the 29 (83%) gastric

cancer patients and 39/58 (67%) controls had antibodies to *H. pylori* (matched odds ratio, 2.1; 95% CI, 0.72–6.4). Exclusion of the three gastric cancer patients with cancers of the cardia and the corresponding controls, justified because of the previously identified specificity of association with cancer other than of the cardia (Nomura *et al.*, 1991; Talley *et al.*, 1991a), resulted in an odds ratio of 2.8 (95% CI, 0.82–9.6) for the patients with cancers at sites other than the cardia. Exclusion of non-cardia gastric cancer patients aged 70 years or over (and corresponding controls), justified because of the previously identified reduced association in the elderly (Nomura *et al.*, 1991), resulted in an odds ratio of 6.0 (95% CI, 1.1–34). Comparisons of cases on the basis of stage or severity of pathological lesions were reported not to affect the odds ratio. [The Working Group noted that the exclusion of patients with known stomach disease from the control group would be likely to reduce the prevalence of *H. pylori* infection in the group and, hence, bias the estimated odds ratio upwards.]

Lin *et al.* (1993b,c) compared 148 gastric adenocarcinoma patients with two series of controls (n = 92 and 823) for the presence of IgG antibodies to *H. pylori*, using a commercial ELISA kit with a reported sensitivity of 96% and a specificity of 93%. The cases were confirmed histologically and occurred in a consecutive series of patients diagnosed in 1992 at a single hospital in Taiwan, China. The mean age was 59 years (range, 24–87 years), and 61% were men. The first control series were part of a group of asymptomatic subjects who had had an endoscopic examination with negative results during a routine health check in 1992. Their mean age was 52 years (range, 22–77 years), and 59% were men. The second control series were randomly selected from household registry files in one precinct and three townships in Taiwan. The subjects included people of all ages, from < 10 years to ≥ 70 years, and 50% were men. [The Working Group noted that the two reports of the study had slightly different numbers of cases: 148 (Lin *et al.*, 1993b) and 143 (Lin *et al.*, 1993c). In the results reported below, the larger number was used, except where stated. The Working Group also noted that the selection of controls for the first series, excluding volunteers who did not have endoscopically normal stomachs, would be likely to reduce the estimated prevalence of *H. pylori* infection in the control group and, hence, bias the estimated odds ratio upwards.] Ninety-two of the 148 (62%) gastric cancer patients and 57/92 (62%) controls in the first series had antibodies to *H. pylori* (age- and sex-adjusted odds ratio, 1.0; 95% CI, 0.59–1.8), as did 448/823 (54%) controls in the second series [unadjusted odds ratio, 1.4 (95% CI, 1.0–2.0); after exclusion of controls from the second series who were aged less than 20 years, 347/527 (65%) were seropositive, giving a calculated unadjusted odds ratio of 0.85 (95% CI, 0.58–1.2)]. Among subjects below the age of 60 years, 44/64 (69%) of gastric cancer cases, 40/66 (61%) of the first series of controls and 280/436 (64%) of the second series of controls (20–59 years) were seropositive; among those 60 years of age or more, 48/84 (57%) of the cancer patients, 17/26 (65%) of the first series of controls and 67/91 (74%) of the second series of controls were seropositive. Twenty-six of the cancer patients had their tumour in the region of the cardia, and 17 of these (65%) were seropositive; 114 cancer patients had their tumour in regions other than the cardia, and 71 of these (62%) were seropositive. Of the 52 patients who had cancers of the intestinal type, 31 (60%) were seropositive, whereas of 96 patients with cancers of the diffuse type, 61 (64%) were seropositive. Of 26 'early' gastric

cancer patients, 16 (62%) were seropositive, and of 122 patients with advanced cancers, 76 (62%) were seropositive.

Kuipers *et al.* (1993c) compared 116 gastric adenocarcinoma patients with 116 age- and sex-matched controls for the presence of IgG antibodies to *H. pylori* using a previously described ELISA (Peña *et al.*, 1989). The cases were confirmed histologically; the patients were resident in the Netherlands and had a median age of 67 years (range, 23–92 years); 56% were men. Controls were subjects undergoing upper gastrointestinal investigations, excluding those with endoscopic and histological abnormalities such as peptic ulcer, atrophic gastritis and intestinal metaplasia. Antibodies to *H. pylori* were found in 89/116 (77%) gastric cancer patients and 92/116 (79%) controls [resulting in an unadjusted and unmatched odds ratio of 0.86 (95% CI, 0.44–1.7)]. Stratification into five age groups (< 50, 50–59, 60–69, 70–79 and > 79 years) did not significantly change the odds ratios for gastric cancer within any strata [figures not available]. Of the 67 gastric cancer patients who had tumours of the intestinal type, 51 (76%) were seropositive; of the 36 patients with tumours of the diffuse type, 28 (78%) were seropositive. [The Working Group noted that, despite the exclusions from the control series, the use of symptomatic gastrointestinal disease patients would be likely to increase the estimated prevalence of *H. pylori* infection among the controls and, hence, bias the odds ratio downwards.]

Estevens *et al.* (1993) compared 80 gastric adenocarcinoma patients with 80 age- and sex-matched controls for the presence of IgG antibodies to *H. pylori* using an ELISA developed in their laboratory on the basis of a previously described assay (Evans *et al.*, 1989). The cases were confirmed histologically and occurred in a consecutive series diagnosed in 1990–91 at a single hospital in Lisbon, Portugal. The mean age was 66 years (SD, 11.9 years), and 58% were men. Controls were blood donors and hospital out-patients attending trauma and orthopaedic clinics. Antibodies to *H. pylori* were found in 56/80 (70%) gastric cancer patients and 65/80 (82%) controls, resulting in an odds ratio of [0.54 (95% CI, 0.24–1.2)]. Of the gastric cancer patients with tumours of the cardia, 67% were seropositive; of the patients with tumours at other sites, 70% were seropositive. Of the patients with tumours of the intestinal type, 64% were seropositive, whereas of those with tumours of the diffuse type, 50% were seropositive.

Archimandritis *et al.* (1993) compared 47 gastric adenocarcinoma patients with 50 controls, matched for age, sex, socioeconomic status and area of residence. The presence of IgG antibodies to *H. pylori* was assessed using a commercial ELISA kit. The cases were confirmed histologically; patients with tumours of the cardia were excluded. Patients were from all over Greece, their mean age was 62 years (SD, 12.6 years) and 62% were men. Controls were healthy people from all over Greece with 'no evidence of peptic ulcer or non-ulcer dyspepsia'; their mean age was 62 years (SD, 14.1 years), and 54% were men. Of the 47 gastric cancer patients, 34 (72%) were seropositive for *H. pylori* antibodies, as were 34/50 (68%) controls (odds ratio, 1.2; 95% CI, 0.51–3.0). When the analysis was stratified by age, the odds ratio for subjects aged < 60 years was 1.5 (0.42–5.0) and that for subjects > 60 was 0.87 (0.23–3.3). Of the 31 gastric cancer patients with tumours of the intestinal type, 22 (71%) were seropositive (1.2; 0.43–3.1); of nine patients with tumours of the diffuse type, seven (78%) were seropositive (0.83; 0.13–5.3). [The Working Group noted that the information provided about control selection was inadequate to allow a judgement about the

adequacy of the control group. The exclusion of controls with peptic ulcer or non-ulcer dyspepsia would be likely to reduce the prevalence of *H. pylori* infection in the control group and, hence, bias the esimated odds ratio upwards.]

The studies are summarized in Table 6.

2.4.2 *Other cancers*

No case–control studies of cancers other than gastric cancer have been reported, although the study of Talley *et al.* (1991a) (see above) compared patients with lung, oesophageal and large bowel cancers.

2.5 Intervention studies

Wotherspoon *et al.* (1993) gave *H. pylori* eradication therapy to six patients (three men, aged 37, 76 and 42, and three women, aged 75, 60 and 57) with histological and molecular genetic evidence of primary gastric low-grade B-cell mucosa-associated lymphoid tissue lymphoma with concomitant *H. pylori* infection. *H. pylori* was eradicated in all six patients, and repeated biopsies, 4–10 months after eradication, in five patients showed no evidence of lymphoma.

Stolte *et al.* (1994a) treated 16 patients with low-grade mucosa-associated lymphoid tissue lymphomas, *H. pylori* infection and gastritis with *H. pylori* eradication therapy. The patients were followed up with repeated endoscopic biopsies 3–12 months after treatment; 12 patients showed regression of the lymphoma. In six of the 12, sparse residual lymphoma tissue was found.

The gastric lymphomas that respond to *H. pylori* eradication therapy, the well-differentiated mucosa-assocated lymphoid tissue lymphomas, were previously called 'pseudolymphomas'. They are known to remain localized for many years before invading other tissues.

3. Studies of Cancer in Experimental animals

3.1 Infection with *Helicobacter pylori* alone

No data were available to the Working Group.

3.2 Infection with *Helicobacter pylori* in combination with administration of known carcinogens

Rat: A total of 90 male Wistar WKY/Std rats, eight weeks of age, received 50 mg/L *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MMNG) in the drinking-water for 40 weeks. One group of 30 rats received MNNG alone; a second group of 30 rats was given MNNG plus oral intubations of 0.2 ml brucella broth three times a week for the 40 weeks; the third group of 30 rats received MNNG and brucella broth containing 10^6–10^8 colony-forming units/ml of culture of fresh isolates of *H. pylori* three times a week for 40 weeks, since permanent

Table 6. Seroprevalence for *Helicobacter pylori* in gastric cancer patients and matched controls: retrospective studies

Country	Cases			Controls			Odds ratio[a]	95% CI	Controls	Reference
	No.	*H. pylori* infection		No.	*H. pylori* infection					
		No.	%		No.	%				
USA	69	36	52	252	96	38	1.6	[0.9–2.8]	Volunteers (76), hospital patients except cancer (176)	Talley *et al.* (1991a)
Finland	54	38	70	84	43	51	[2.3	1.0–5.0]	Cancer patients except gastric	Sipponen *et al.* (1992)
Republic of Korea	28	25	89	30	20	67	4.2	1.0–17	Hospital patients	Kang & Chung (1992)
Sweden	112	90	80	103	63	61	2.6	1.4–5.0	Hospital patients	Hansson *et al.* (1993a)
Japan	29	24	83	58	39	67	2.1	0.72–6.4	Hospital out-patients	Blaser *et al.* (1993)
China (Taiwan)	148	92	62	92	57	62	1.0	0.59–1.8	Health check-up participants	Lin *et al.* (1993b)
Netherlands	116	89	77	116	92	79	[0.86]	[0.44–1.7]	Gastroenterology patients except ulcer, gastritis	Kuipers *et al.* (1993c)
Portugal	80	56	70	80	65	81	[0.54]	[0.24–1.2]	Blood donors, hospital out-patients	Estevens *et al.* (1993)
Greece	47	34	72	50	34	68	1.2	0.51–3.0	Healthy people	Archimandritis *et al.* (1993)

CI, confidence interval

[a]From primary analysis reported in paper, using all cases of gastric cancer

colonization of the rat gastric mucosa by the *H. pylori* is not achieved. All rats survived 35 or more weeks. After the 40 weeks of treatment, the two control groups had very similar numbers of gastroduodenal tumours (adenomatous polyps, adenocarcinomas and carcinomas): 7/30 of those given MNNG alone and 6/30 of those given MNNG plus brucella broth; a slight reduction in the number of gastroduodenal tumours was seen in the group given MNNG plus the living cultures of *H. pylori* (4/30). No difference in the incidence of gastritis was seen among the three groups (Kawaura *et al.*, 1991). [The Working Group noted that exposure to *H. pylori* was intermittent in this model, thus unlike the conditions of human exposure.]

3.3 Infection with other *Helicobacter* species

Mouse: In a study reported as an abstract (Enno *et al.*, 1994), 260 specific pathogen-free BALB/c mice were infected with *H. felis*. Groups of 20 mice were killed at 2–3-month intervals up to 26 months. Up to 18 months after infection, minimal gastritis was observed; however, at 22–26 months after infection, 51/80 *H. felis*-infected animals and 4/48 uninfected controls had large lymphoid aggregates in the cardia. Lymphoepithelial lesions that were not seen in control animals and which, according to the authors, are similar to those observed in association with human gastric low-grade B-cell lymphomas, were observed in 27/80 infected animals.

3.4 Infection with other *Helicobacter* species in combination with administration of known carcinogens

Ferret: A group of nine female ferrets (*Mustela putorius furo*), four to five months of age, ovariohysterectomized and naturally infected with *H. mustelae*, received single oral doses of 50 mg/kg bw MNNG in 3 ml of olive oil. One additional four-month-old ferret received 100 mg/kg bw MNNG, and five controls animals received olive oil only. Mucosal punch biopsies were obtained by endoscopy from the same region of the stomach at 6–12-month intervals; no adenocarcinoma was seen in the limited samples taken. Seven of the nine ferrets dosed with 50 mg/kg bw MNNG were killed between 51 and 55 months after treatment; one other ferret died, and one was killed at 25 months. At necropsy, two ferrets had pyloric ulcers and two had obvious nodules on the mucosal surface of the pylorus. The single ferret that received 100 mg/kg bw and was killed at 29 months had clinical gastrointestinal disease. It had a grossly thickened pyloric area with a 1-cm ulcer at the pyloric–duodenal junction. Histopathological examination of all the stomachs revealed that all ferrets, control and treated, had marked chronic gastritis with the major characteristics of multifocal atrophic gastritis. One or more foci of neoplasia were seen in 9 of the 10 MNNG-treated ferrets. Two had well-defined invasive adenocarcinomas, and four had multiple independent primary adenocarcinomas. The neoplasms were concentrated in the pyloric antrum at the transition zone between the corpus and antral mucosa. Metastasis to regional lymph nodes was observed in one animal. The five control animals were killed 47–67 months after dosing with olive oil; two that were killed had chronic renal failure, while the other three were asymptomatic when they were killed. No gross lesion was seen in the stomachs of the control ferrets; the only histopathological change observed was mild to moderate gastritis in the

antrum with small foci of gland loss. Adenocarcinomas were not observed in the stomachs of hundreds of untreated laboratory ferrets examined at routine necropsy (Fox *et al.*, 1993a). [The Working Group noted that the study did not include a group uninfected with *H. mustelae* but given MNNG.]

4. Other Data Relevant for Evaluation of Carcinogenicity and its Mechanisms

4.1 Pathology of infection

Cross-sectional and longitudinal observations in human populations indicate that a series of alterations of the gastric mucosa precede gastric carcinoma (Siurala *et al.*, 1985; Correa *et al.*, 1990b; Kuipers *et al.*, 1994a): They follow a sequential presentation of chronic nonatrophic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia. Atrophy (loss of gastric glands) is a pivotal change in the precancerous process. It radically alters the gastric microenvironment by reducing acid secretion, elevating the gastric luminal pH and resulting in an overgrowth of anaerobic bacteria. Many such bacteria produce reductases which act on nitrate molecules (from food and other sources) and result in elevated concentrations of NO_2^- in the gastric lumen. Dietary factors that are important in the progression of the precancerous process include high salt (NaCl) intake and low consumption of fresh fruits and vegetables (Nomura *et al.*, 1982; Fontham *et al.*, 1986; Buiatti *et al.*, 1989b, 1990; Chen *et al.*, 1990; Forman, 1991).

4.1.1 *Humans*

The anatomical substratum resulting from *H. pylori* infection is chronic gastritis. Although the association between the bacterium and gastritis was recognized only in 1983 (Warren, 1983; Marshall, 1983), the pathological manifestations of chronic gastritis and several nosological entities of gastritis had been described previously.

(a) Specific lesions

Colonies of *H. pylori* are characteristically located extracellularly in the mucus layer immediately adjacent to the gastric surface epithelium. They are prominently concentrated in front of the intercellular junctions of the epithelial cells. Most bacteria float freely within the mucus layer; a few adhere to pedestals formed by the epithelial cytoplasmic membrane. They may sometimes penetrate the intercellular spaces and, rarely, the ductules of the parietal cells (Chen *et al.*, 1986; Fiocca *et al.*, 1987; Hessey *et al.*, 1990).

H. pylori infection is associated with degenerative changes in the cytoplasm of the surface epithelial cells, identified on haematoxylin–eosin staining as loss of the superficial portion of the cytoplasm, resulting in microerosions of the surface epithelium (Chan *et al.*, 1991). Under the electron microscope, partial loss and stunting of the microvilli and numerous intracellular phagolysosomes may be seen (Chen *et al.*, 1986; Fiocca *et al.*, 1987; Hessey *et al.*, 1990).

H. pylori infection results in infiltration of leukocytes into the gastric mucosa. The most abundant are B lymphocytes, which occupy the lamina propria and may lead to formation of

lymphoid follicles (Genta *et al.*, 1993a,b). Polymorphonuclear neutrophils, although less abundant than lymphocytes, are common in *H. pylori* infections. They are seen in the lamina propria, in the space between the epithelial cells and in the gastric lumen; they typically aggregate in the neck area of the gastric glands. Other inflammatory cells identified in *H. pylori*-infected mucosa are plasma cells, T lymphocytes, macrophages and eosinophils (Marshall *et al.*, 1985b; Dixon *et al.*, 1988; Wyatt & Rathbone, 1988; Genta *et al.*, 1993b).

The lesions associated with acute (new) infection are similar to those described above, except that the polymorphonuclear infiltrate is prominent and precedes the lymphocytic infiltrate (Marshall *et al.*, 1985a; Morris & Nicholson, 1987; Graham *et al.*, 1988).

(*b*) *Nosological entities*

In early stages of *H. pylori* infection, gastritis is nonatrophic. Later, it leads to gland loss (atrophic gastritis) and is frequently followed by intestinal metaplasia.

Atrophic gastritis in patients with the pernicious anaemia syndrome diffusely involves the oxyntic mucosa while sparing the antrum. This gastritis is called type A or autoimmune (Strickland & Mackay, 1973; Correa, 1980). In populations at low risk for pernicious anaemia, atrophic gastritis is multifocal and involves both the antrum and the corpus. This gastritis is called type B (Strickland & McKay, 1973) or multifocal atrophic (Lambert, 1972; Correa, 1980). The two entities coexist in a few patients, leading to the denomination type AB gastritis (Glass & Pitchumoni, 1975). A frequent form of nonatrophic gastritis is located predominantly in the antrum, with mild or no involvement of the oxyntic mucosa. Such lesions have been called diffuse antral (Correa, 1988), interstitial (Cheli *et al.*, 1980), pre-atrophic (Cheli & Testing, 1993) or hypertrophic gastritis (Schindler, 1969). This type of gastritis is seen most frequently in conjunction with duodenal ulcer, while multifocal atrophic gastritis is associated particularly with gastric ulcer or gastric carcinoma (Schindler, 1969; Lambert, 1972).

Once the prominent role of *H. pylori* in chronic gastritis had been recognized, a grading of gastritis, the Sydney system, was designed (Price, 1991), which is intended to include microscopic, gastroscopic and etiological factors, including *H. pylori* infection. The system allows the grading of inflammatory and atrophic changes in the corpus and antrum on a semiquantitative scale of 0–3. The name 'pangastritis' is proposed for lesions covering both the antrum and the corpus, which can be atrophic or nonatrophic (Sipponen *et al.*, 1991).

H. pylori infection has a prominent role in diffuse antral (nonatrophic) gastritis and in multifocal atrophic gastritis (Siurala *et al.*, 1985). It has no role in corpus-limited (type A or autoimmune) atrophic gastritis, or in other specific forms of gastritis such as those associated with bile reflux or use of nonsteroidal anti-inflammatory drugs, known as 'reflux', 'reactive' or 'chemical irritational' gastritis (Dixon *et al.*, 1988; Flejou *et al.*, 1989), or in 'lymphocytic gastritis' (Haot *et al.*, 1986).

H. pylori infection has also been associated with other, less frequent types of gastritis, such as that characterized by prominent hyperplastic foveola, also called 'hypertrophic' or 'focal foveolar' hyperplasia (Stolte *et al.*, 1994b).

(*c*) *Epidemiology of chronic gastritis*

H. pylori infection is very prevalent in some populations of low socioeconomic status (Holcombe, 1992; Sierra *et al.*, 1992). In a few, gastric biopsy specimens and pepsinogen levels indicate that the gastritis is not of the atrophic type (Sierra *et al.*, 1992; Shousha *et al.*, 1993). In populations at high risk for gastric cancer, atrophic forms of gastritis predominate. Atrophic gastritis associated with the pernicious anaemic syndrome, not usually related to *H. pylori* infection, is strongly related to genetic susceptibility and affects mainly populations of northern European extraction. In other populations at high risk for gastric cancer, such as those of the Andean regions of Latin America, those of China and Japan, and US blacks, atrophic gastritis is multifocal and linked in part to dietary factors (Fontham *et al.*, 1986; Nomura *et al.*, 1982).

People of each sex are equally affected, and the prevalence of gastritis is highly age-dependent. Nonatrophic gastritis is more frequent in people under the age of 50, whereas atrophic gastritis and intestinal metaplasia are more frequent among people over that age (Siurala *et al.*, 1985). In samples from 500 blood donors in Finland, the prevalences of both *H. pylori* antibodies (IgG class in particular, but also IgA and IgM) and gastritis were shown to increase with age (Kosunen *et al.*, 1989).

(*d*) *Relation of infection to gastritis*

The first demonstration of an association between *H. pylori* infection and human disease was the result of two experiments in which *H. pylori* organisms were ingested voluntarily. Acute gastritis was seen in biopsy specimens from both subjects (Marshall *et al.*, 1985a; Momms & Nicholson, 1987), and one of the volunteers developed chronic gastritis. An epidemic of hypochlorhydric gastritis (epidemic achlorhydria) described in 1979 was later shown to be due to transmission of *H. pylori* infection via endoscopy. Acute granulocytic gastritis, lasting some weeks, developed into chronic gastritis within 74 days to two years in these cases (Ramsey *et al.*, 1979; Graham *et al.*, 1988). Successful treatment of *H. pylori* infection leads to healing of gastritis (Rauws *et al.*, 1988; Valle *et al.*, 1991; Kosunen *et al.*, 1992; Genta *et al.*, 1993a).

A positive relationship exists between *H. pylori* infection and gastritis, i.e. with regard to the degree of mucosal inflammation by mononuclear inflammatory cells, polymorphonuclear neutrophils and eosinophils, particularly in the antrum (Stolte *et al.*, 1990; Satoh *et al.*, 1991; McGovern *et al.*, 1991; Stolte *et al.*, 1994b). Specific cytotoxic strains are shown to enhance the inflammatory response, and their occurrence differs between populations.

In a random sample of gastric biopsy specimens from the antrum, corpus or both in Finland, up to 91% of people with nonatrophic (superficial) gastritis, up to 41% with advanced atrophic gastritis but none with normal stomachs or severe atrophic gastritis of the autoimmune (type A, or corpus-limited) type contained *H. pylori* (Siurala *et al.*, 1988). In a subset of patients with advanced atrophic gastritis, the estimated prevalence of *H. pylori* infection was higher when assessed by both serological and histological methods than when it was assessed by histology alone (Karnes *et al.*, 1991). In populations at high risk for gastric cancer, such as in Colombia, the prevalence of *H. pylori* is close to 100% (Correa *et al.*, 1989).

(*e*) *Atrophic gastritis and intestinal metaplasia*

In a 3–16-year (average, 5.1 years) follow-up study (7290 person-years) of people in Narino, Colombia, the rate of transition from normal histological appearance or superficial gastritis to atrophic gastritis or more advanced lesions was 3.3% per year, corresponding to 1.7% for atrophic gastritis, 0.9% for intestinal metaplasia and 0.7% for dysplasia (Correa *et al.*, 1990b).

Mathematical modelling of cross-sectional data on gastritis in Finland and Estonia indicated a slow, stepwise transition from nonatrophic gastritis to atrophic gastritis over time (Kekki & Villako, 1981; Kekki *et al.*, 1983). The fractional transition rate from the pool of nonatrophic to the pool of atrophic gastritis was estimated to be 2.1–2.6% per year for people aged 25–75 (Kekki & Villako, 1981; Villako *et al.*, 1982).

In an 11.5-year (range, 10–13) follow-up of 113 patients with and without *H. pylori* gastritis in the Netherlands, significant progression of nonatrophic gastritis to atrophic gastritis was demonstrated endoscopically (Kuipers *et al.*, 1994a). Fifteen of 56 (27%) patients with *H. pylori* infection and nonatrophic gastritis developed atrophic gastritis, whereas only two of 49 (4%) patients without *H. pylori* infection, all of whom had normal gastric mucosa at the beginning of follow-up, developed the atrophic stage. The difference was significant ($p < 0.001$).

An endoscopic follow-up of 377 subjects in Finland for 30–34 years (Ihamäki *et al.*, 1985) revealed that progression of atrophic gastritis occurs in the gastric corpus and regression may occur in the antrum in the long term.

Since nonatrophic gastritis involves predominantly the antrum and multifocal atrophic gastritis compromises to a large degree both the antrum and the corpus, it is important to study the dynamics of involvement of *H. pylori* in different regions of the stomach. The location and severity of gastritis vary in different disease manifestations of *H. pylori* infection. Thus, inflammation in duodenal ulcer patients is generally restricted to the antrum, while in those with gastric ulcer and gastric cancer the gastritis is more widely distributed in the corpus of the stomach (Stolte *et al.*, 1990). Observations on patients with different acid outputs may be relevant. In patients given the anti-acid secretory drug omeprazole, gastritis in the antrum is reduced, while inflammation in the corpus increases, i.e. pangastritis is observed (Solcia *et al.*, 1994). In a study on the long-term effects of omeprazole in 91 patients, only 1% had atrophic gastritis at the beginning of therapy, but on follow-up (mean, 48 months; range, 36–64 months), 25% had atrophic gastritis (Klinkenberg-Knol *et al.*, 1994). Other studies of prolonged omeprazole treatment show lower rates of transition to atrophic gastritis (Lambert *et al.*, 1993).

Studies conducted before identification of *H. pylori* also showed changes in the distribution and intensity of gastritis after acid suppression. After vagotomy, a surgical procedure to reduce acid output in duodenal ulcer patients, a marked increase in both the extent and severity of proximal gastritis was seen, but the distal gastritis remained unchanged (Meikhle *et al.*, 1976).

The development of atrophic gastritis depends on factors in addition to *H. pylori* infection (Correa, 1992; Fukao *et al.*, 1993). Genetic susceptibility to atrophic gastritis was seen in segregation analysis in Narino, Colombia, suggesting that expression of a single

autosomal recessive gene, with age-dependent penetrance, is involved (Bonney *et al.*, 1986; see also section 1.1.2(*b*)).

(*f*) *Atrophic gastritis and gastric cancer*

In a meta-analysis of six independent follow-up studies (Varis, 1983), 58 cases of gastric cancer (severe corpus-limited atrophic gastritis) were recorded among 843 patients with pernicious anaemia who were followed up for 7.8–15 years (mean, 11 years; 8990 person-years), providing an estimate of 0.6% for the annual cancer risk and suggesting that the occurrence of cancer is approximately five times higher among patients with severe atrophic corpus gastritis than in the population at large. An 11–14-year follow-up of three population samples in Finland (over 800 people) with and without gastritis indicated that the risk for developing gastric cancer was two to three times higher than that expected in people who had advanced atrophic gastritis. All 10 patients with gastric malignancy had had gastritis at the beginning of follow-up, and none without it developed advanced disease (Ihamäki *et al.*, 1991).

Estimates of cancer risk in association with multifocal atrophic gastritis have been based on the results of case–control studies (Sipponen *et al.*, 1985, 1994a), which suggest that the age- and sex-adjusted relative risk for gastric cancer is increased by up to 18 fold. The risk rises to 90 fold in patients with severe pangastric atrophy (Sipponen *et al.*, 1985).

The risk for gastric cancer and, in particular, intestinal-type gastric cancer, is increased in the presence of intestinal metaplasia and atrophic gastritis (Correa, 1992; Sipponen *et al.*, 1992), especially if the intestinal metaplasia is of type III (Jass & Filipe, 1980), also called the colonic or incomplete type (Jass & Filipe, 1979; Jass, 1980; Sipponen *et al.*, 1980). Precancerous lesions of various types and nature (polyps, dysplasia) have been shown to be associated with atrophic gastritis and intestinal metaplasia (Laxén *et al.*, 1983; Correa *et al.*, 1990b). The risk for gastric cancer associated with different types of intestinal metaplasia was investigated in a cohort of 1525 Slovenian patients. The standardized incidence ratio for stomach cancer was 2.2. When type I metaplasia was used as the reference category, the risk was 2.1 for type II and 4.6 for type III (Filipe *et al.*, 1994).

There is some evidence of a relationship between the occurrence of intestinal metaplasia and atrophic gastritis and tumours at the same anatomical site in the stomach (Sipponen *et al.*, 1983).

(*g*) *Nonatrophic gastritis and gastric cancer*

In a case–control study, the age- and sex-adjusted risk for gastric cancer was slightly but significantly increased (two to three fold) in patients with nonatrophic gastritis over that in subjects with normal, uninfected stomachs (Sipponen *et al.*, 1994).

(*h*) *Mucosal-associated lymphoid tissue*

B-Cell lymphoid follicles and aggregates resembling intestinal Peyer's patches appearing mainly in the gastric antrum and small curvature of the stomach are a characteristic feature of *H. pylori*-related gastritis; they represent acquired mucosa-associated lymphoid tissue in the stomach (Isaacson, 1992). These follicles do not occur in uninfected subjects or in special forms of gastritis (Stolte & Eidt, 1989; Genta *et al.*, 1993a), whereas they

have been reported to occur in 27–100% of cases with *H. pylori*-related gastritis (Genta *et al.*, 1993a). Their prevalence increases with the degree of inflammatory reaction (Stolte & Eidt, 1989). Treatment of *H. pylori* infection results in a slow decrease (but not the disappearance) of lymphoid follicles within 12 months (Genta *et al.*, 1993b).

In the most comprehensive study, which was designed to determine the frequency and distribution of gastric lymphoid follicles in *H. pylori* infection, mapped gastric biopsy specimens were obtained from 20 normal, uninfected volunteers, 25 asymptomatic volunteers with *H. pylori* infection and no ulcer disease, 21 duodenal ulcer patients, and 16 patients with gastric ulcer. None of the uninfected patients had lymphoid follicles, while all subjects infected with *H. pylori* had follicles. Eradication of the organism with antimicrobial agents resulted in a slow decrease in the prevalence of follicles (Genta *et al.*, 1993a).

4.1.2 *Experimental systems*

Investigation of animals infected with different *Helicobacter* species provides the opportunity to confirm the role of these bacteria in chronic gastritis and also to demonstrate the progression of chronic gastritis to atrophic gastritis.

(*a*) *Non-human primates*

Many studies have shown that a number of primate species are colonized with bacteria similar to *H. pylori*. In a closed colony of rhesus monkeys (*Macaca mulatta*), chronic gastritis was found in 8 of 11 animals surveyed, and inflammation was correlated with the presence of *H. pylori*-like bacteria (Baskerville & Newell, 1988). The inflammatory infiltrate was primarily mononuclear, and the lamina propria was heavily infiltrated by lymphocytes, plasma cells and histiocytes. Large lymphoid follicles occurred in most stomachs. Polymorphonuclear leukocytes were rarely seen. When intense cellular infiltration was present in the body of the stomach, atrophy of glands containing parietal and chief cells was observed.

Examination of another rhesus monkey colony revealed marked abnormalities in a number of animals (Euler *et al.*, 1990). There was a noticeable mixed mononuclear cell inflammatory response in 14/35 animals examined. *H. pylori* was cultured from 12/35 animals. A strong correlation was seen with gastritis: inflammation occurred in 83% of infected animals and in only 17% of uninfected animals. When two groups of five uninfected monkeys without gastritis at the time of screening were inoculated experimentally with either human or monkey isolates of *H. pylori*, the human strain did not colonize the animals, but all of them became infected with the monkey isolate and all had gastritis by 28 days after inoculation.

Dubois *et al.* (1991) found *H. pylori*-like bacteria in 8 of 29 colony-bred rhesus monkeys, and all had gastritis; however, of 14/29 infected with '*H. heilmanni*', only two had gastritis. Uninfected animals had no gastritis.

The Japanese monkey (*Macaca fuscata*) has also been used as an experimental model (Shuto *et al.*, 1993). Of 12 animals inoculated with a human isolate of *H. pylori*, seven became infected and inflammation characterized by polymorphonuclear leukocytes and monocytes was observed. *H. pylori*-associated gastritis persisted in two animals followed for more than 18 months.

(*b*) *Gnotobiotic piglets*

Krakowka *et al.* (1987) were able to infect 17 gnotobiotic domestic Yorkshire piglets with a human isolate of *H. pylori*. Histopathological lesions indicative of chronic active gastritis were seen in all infected piglets. A neutrophilic response was present for two weeks but then resolved, and the gastritis consisted primarily of mononuclear cells and prominent lymphoid follicles. As piglets can be maintained in the gnotobiotic state for only six weeks, the progression of gastritis could not be assessed.

In a further study, seven pigs were immunized with 10^9 *H. pylori* in incomplete Freund's adjuvant in two doses given subcutaneously seven days apart; these pigs and eight un-immunized control pigs were then infected with a human strain of *H. pylori*. The gastritis was much more severe in the previously immunized than in the unimmunized piglets. Neutrophilic infiltrates and neutrophilic gland abscesses were seen in the immunized but not in the unimmunized piglets (Eaton & Krakowka, 1992).

(*c*) *Dogs*

H. pylori has been shown to infect gnotobiotic, germ-free beagle puppies, and significant chronic gastritis was induced in all infected animals (Radin *et al.*, 1990). A more intense gastritis was induced when the pups were inoculated with pure cultures of *H. felis*, an organism commonly seen in dogs (Lee *et al.*, 1992). All infected dogs showed extensive mononuclear inflammation, with the appearance of large lymphoid aggregates. As the animals were kept for only 30 days after infection, no progression of gastritis was observed.

(*d*) *Cats*

When kittens were infected with either *H. acinonyx*, a species of *Helicobacter* isolated from a group of cheetahs with gastritis, or '*H. heilmannii*', a *Helicobacter*-like bacterium found in the same groups of cheetahs, both organisms colonized the feline stomachs and induced a mild lymphofollicular gastritis, which did not change over 11 months (Eaton *et al.*, 1993).

A closed colony of cats bred by a commercial vendor was shown to be infected by *H. pylori*. The bacterium colonized primarily the antrum and induced antral gastritis (Handt *et al.*, 1994).

(*e*) *Ferrets*

In a study in which 11 adult ferrets were extensively examined (Fox *et al.*, 1990), *H. mustelae* was present in all animals, and a diffuse antral gastritis similar to that seen in humans infected with *H. pylori* was observed. In some animals, the changes observed in the proximal antrum and the transitional zone appeared to be similar to the early stages of multifocal atrophic gastritis in humans.

(*f*) *Rodents*

Rodents have not been shown convincingly to become colonized with *H. pylori*; however, the feline *Helicobacter*, *H. felis*, readily colonizes both rats and mice for the life of the animal (Lee *et al.*, 1993). When four-week-old female Swiss-Webster, isolator-reared, axenic mice were given viable *H. felis* orally (Lee *et al.*, 1990), 18/20 mice became infected. The first

evidence of gastritis was seen two weeks after inoculation and was mainly neutrophilic; by four weeks, the severity of inflammation had increased and there were more lymphocytes. By eight weeks, all mice had a relatively diffuse active chronic gastritis, with a cell infiltrate composed of approximately equal numbers of mononuclear and polymorphonuclear leukocytes, with lymphocytes and neutrophils as the predominant cell types. Small lymphoid nodules had formed in the submucosa, and small aggregates of lymphocytes in the subglandular area displaced or compressed mucosal glands. In a more extensive study, the course of gastritis was followed up to 50 weeks after infection (Fox *et al.*, 1993b). Between 20 and 50 weeks, the gastritis became more chronic, although microabscesses were seen in some animals even at this late stage. A similar study in rats showed the induction of chronic gastritis that was less florid than that in the mice (Fox *et al.*, 1991).

The only long-term animal study that allows assessment of the severity of gastritis over the life of infected animals is one in conventional Quackenbush Swiss mice (Lee *et al.*, 1993). A total of 221 seven-week-old female mice were infected with either a living culture of *H. felis* or a gastric homogenate from mice infected with '*H. heilmannii*'. The severity of gastritis was assessed in mice killed at regular intervals for up to 72 weeks. All infected mice showed a slowly progressive chronic gastritis, with increasing numbers of infiltrating mononuclear cells and polymorphonuclear leukocytes. After a year and a half, the inflammatory reaction was so severe that atrophic changes were seen in both the antral and fundic mucosa. Control animals initially showed no inflammatory changes; however, as the animals aged, the gastric mucosa of some animals became infected with a bacterium, *H. muridarum*, that normally inhabits the small and large bowel of the rodent. The presence of this bacterium was also associated with gastritis and atrophic changes.

A severe, long-term gastritis was shown in mice infected for more than six months with an '*H. heilmannii*', *Helicobacter*-like organism originating from a cheetah that had gastritis (Eaton *et al.*, 1993). The infected mice had grossly evident gastric mucosal hypertrophy at sacrifice, with severe lymphoplasmacytic inflammation, lymphoid follicles and microscopic ulcers.

Mice infected with another animal *Helicobacter*, '*Gastrospirillum suis*' from pigs, also developed gastritis (Moura *et al.*, 1993). Some degree of glandular destruction in the oxyntic mucosa due to an inflammatory reaction involving granulocytes and mononuclear cells was described.

4.2 Other observations relevant to the interpretation of carcinogenicity and mechanisms of carcinogenesis

4.2.1 *Humans*

H. pylori may act in the development of gastric cancer by a number of possible mechanisms: (i) an increase in the rate of epithelial cell proliferation; (ii) damage to mucus secretion and the cytoplasm of foveolar cells; (iii) facilitation of the synthesis and delivery of carcinogens at the site, especially *N*-nitroso compounds; (iv) inhibition of the local effect of antioxidants, especially L-ascorbic acid; and (v) induction of mutations and other molecular lesions, either directly or through the release of active oxygen species and $NO^{\bullet}$ by polymorphonuclear cells and macrophages attracted by the bacteria.

(*a*) *Increased cell replication*

Atrophic gastritis increases the rate of proliferation of the gastric epithelium (Lipkin *et al.*, 1985), as measured by tritiated thymidine incorporation. This effect was found to be associated with *H. pylori* infection (Buset *et al.*, 1992; Cahill *et al.*, 1993; Fischbach *et al.*, 1993) in patients with multifocal atrophic gastritis and infected with *H. pylori*. Gastric biopsy specimens taken from patients before and after therapy for *H. pylori* infection were immunostained with antibodies against the proliferating cell nuclear antigen (Brenes *et al.*, 1993). In patients who cleared the infection, the labelling index was reduced from 19.95 to 14.12 ($p < 0.001$), close to the normal index of 13.05. Patients who did not clear the infection showed no reduction in labelling index (18.9 before and 17.9 after treatment). Hyperproliferation of the gastric epithelium thus appears to be caused by *H. pylori* infection.

Both cell proliferation and ploidy have been assessed on the basis of the nucleolar organizer regions. The number of regions is increased in the gastric epithelium of patients infected with *H. pylori*, but after successful treatment the region count is rapidly reduced to normal levels (Correa *et al.*, 1994).

(*b*) *Alteration of the mucus barrier*

This mechanism is presumed to be important because the gastric microenvironment of atrophic gastritis patients contains concentrations of NO_2^- and nitrogen-containing species that can produce carcinogens but may be separated from the target cell by a normal mucus barrier. The gastric epithelium is thus protected from the acid environment in the gastric lumen by complex mucus glycoproteins. *H. pylori* organisms produce proteases and lipases which degrade the mucus gel, causing loss of hydrophobicity (Goggin *et al.*, 1992; Go *et al.*, 1993) and viscosity, which induces breaks in the continuity of the mucus layer (Sidebotham *et al.*, 1991; Slomiany & Slomiany, 1992). This change is followed by increased production of prostaglandin E2 (Oderda *et al.*, 1993). The damage to the mucus is also associated with bile reflux, a common finding in *H. pylori*-associated gastritis (Sobala *et al.*, 1991).

(*c*) *Facilitation of synthesis of carcinogens* in situ

There is an extensive literature on the possible generation of *N*-nitroso compounds by overgrowing bacteria in the stomachs of patients with atrophic gastritis (Hill, 1986; Correa, 1992). Substrates involved in this process may be nitrogen-containing compounds in foods, which can react with nitrite to produce carcinogenic and mutagenic *N*-nitroso compounds. Examples include indole substances in fava beans (Yang *et al.*, 1984) and Chinese cabbage (Wakabayashi *et al.*, 1985), which are frequently consumed by inhabitants in areas of high risk for stomach cancer. In Costa Rican schoolchildren, *N*-nitrosoproline excretion after proline intake, measured as a marker of endogenous nitrosation, was slightly higher (about 1.5-fold) in an area of high gastric cancer risk than in a low-risk area (Sierra *et al.*, 1993), although *H. pylori* infection is very prevalent (around 70%) in both high- and low-risk areas. These results indicate either that *H. pylori* infection is not causally related to nitrosation or that nitrosation is selectively inhibited in the low-risk area. As *H. pylori* infection is also prevalent in other areas of low risk for stomach cancer, such as in Africa, other environmental, social and genetic factors appear to be involved in the etiology of gastric cancer (Holcombe, 1992).

H. pylori contains alcohol dehydrogenase but not aldehyde dehydrogenases. The bacterium can thus produce acetaldehyde from even low (0.1%) concentrations of ethanol (Salaspuro, 1994). Acetaldehyde is a highly reactive, toxic substance which has been classified as possibly carcinogenic to humans by an IARC working group (IARC, 1987).

(*d*) *Decreased levels of* L-*ascorbic acid*

Infection with *H. pylori* interferes with the normal capacity of the gastric mucosa to concentrate ascorbic acid. This conclusion is inferred from the fact that uninfected patients have a higher concentration of ascorbic acid in the gastric juice than infected patients (Sobala *et al.*, 1989; Rood *et al.*, 1994); furthermore, previously infected patients can concentrate ascorbic acid at near normal levels after successful antimicrobial therapy (Sobala *et al.*, 1993; Ruiz *et al.*, 1994).

(*e*) *Induction of mutations*

H. pylori has no direct mutagenic activity, and reports of differences in the mutagenicity of gastric juice from patients with and without gastritis are equivocal (Montes *et al.*, 1979; Morris *et al.*, 1984; O'Connor *et al.*, 1984; Farinati *et al.*, 1989). Investigations of alterations in the *p53* gene in 10 gastric adenomas and one carcinoma, however, showed that three of the adenomas contained *p53* mutations (Tohdo et al., 1993). In a study of samples obtained by gastrectomy from 12 patients with gastric cancer in Italy, mutations of the *p53* gene were found in 3/12 normal areas of the stomach, 4/8 areas of metaplasia, 8/12 areas of dysplasia and 9/12 of the carcinomas. In five of seven of the samples that were analysed further, the mutations were shown to be GC→AT transitions in exons 5–8 (Shiao *et al.*, 1994). Amplification of the C-*erb*B.2 gene is related to invasion and nodal involvement. Differential expression of the ras oncoprotein in diffuse-type and in poorly differentiated intestinal-type gastric carcinomas implies that there are two distinct subtypes of gastric carcinoma (Tehara, 1993).

It has been proposed that the activation of polymorphonuclear leukocytes that occurs in the gastritis induced by *H. pylori* could result in the production of oxygen and nitrogen radicals (e.g. hydroxy radicals, nitric oxide), which induce DNA damage (Wink *et al.*, 1991; Nguyen *et al.*, 1992). Davies *et al.* (1994) reported that gastric biopsy specimens from *H. pylori*-infected subjects show more production of reactive oxygen metabolites than specimens from uninfected individuals. An inducible form of nitric oxide synthetase was detected immunohistochemically in epithelial cells of the stomach infected with *H. pylori* in subjects with chronic atrophic gastritis (Pignatelli *et al.*, 1994). [The Working Group noted the inadequate reporting of the data.]

(*f*) *Cytotoxin and cytotoxin-associated protein*

Only one, variable property of *H. pylori* has been shown to be correlated with the severity of disease. It is the vacuolating cytotoxin, first described by Leunk *et al.* (1988), who showed that broth-culture filtrates induced intracellular vacuolation in seven of nine mammalian tissue culture cell lines tested. This toxin was later found to be an 87-kD protein with partial homology with the internal sequences of ion channel proteins (Cover & Blaser, 1992).

Bacterial culture filtrates from a cytotoxin-producing strain of *H. pylori* were incubated with cell cultures. After 16 h, cells were harvested and the Na^+/K^+-ATPase activity was

measured. An immediate reduction in enzyme activity was observed. Filtrates of a non-cytotoxin-producing strain did not inhibit enzyme activity (Ricci *et al.*, 1993).

Soon after identification of the cytotoxin, it was shown that a greater proportion of strains isolated from duodenal ulcer patients were toxigenic (66.6%) than strains isolated from asymptomatic patients (30.1%) (Figura *et al.*, 1989). Also, patients with duodenal ulcer were more likely to have antibodies that neutralize the activity of the toxin in their serum than asymptomatic patients (Pereira Lage *et al.*, 1993).

The sera from all six gastric carcinoma patients and three of five sera from peptic ulcer patients showed neutralizing activity to the cytotoxin, and 21 of 22 stored sera from gastric cancer patients also showed neutralizing activity (Hirai *et al.*, 1994). [The Working Group noted the small number of sera in the first part of this study and the lack of control sera from non-cancer patients in the retrospective study.]

In a study of 30 *H. pylori* patients, 47% of the infecting strains were toxin producers. Cytotoxin production *in vitro* was shown to be associated with increased antral mucosal polymorphonuclear leukocyte infiltration (Cover *et al.*, 1993). Sixty-nine percent (18/26) of strains of *H. pylori* isolated from patients with diffuse antral gastritis and 89% (70/79) of strains isolated from patients with chronic atrophic gastritis were toxin producers ($p = 0.043$) (Fox *et al.*, 1992).

Antibodies against an *H. pylori* 120-kDa protein were found in gastric biopsy specimens from patients infected with *H. pylori*. The presence of the antibody was correlated strongly with the presence of peptic ulcer and severe gastritis (Crabtree *et al.*, 1991). This very immunogenic protein is expressed in association with the vacuolating toxin (Crabtree *et al.*, 1992), and the antigen has been named cagA (cytotoxin-associated protein); its gene (*cag*A) has been sequenced. Clinical isolates that do not produce the antigen do not have the gene and are unable to produce an active vacuolating cytotoxin (Covacci *et al.*, 1993). An ELISA for the 120-kDa protein on sera has allowed investigations of the sera of *H. pylori*-infected patients (Crabtree *et al.*, 1992).

Crabtree *et al.* (1993a) examined the systemic IgG response to *H. pylori* in 70 gastric cancer patients; 79% were seropositive by ELISA for *H. pylori* infection. Of these ELISA-positive sera, 91% recognized the *H. pylori* 120-kDa cagA protein by western blotting, significantly more than a control group of 47 ELISA-positive patients with non-ulcer dyspepsia (72%).

Cytotoxic strains that express the cagA antigen of *H. pylori* have also been shown to induce rapid secretion of significantly more IL-8 in gastric epithelial cell lines than non-cytotoxic strains (Crabtree *et al.*, 1994). IL-8 has been shown to be expressed *in vivo* in *H. pylori*-infected people and is known to be a potent neutrophil chemotactic and activating factor (Crabtree *et al.*, 1993b; Noach *et al.*, 1994). Increased IL-8 production has also been seen in neoplastic tissue. In a further study using immunofluorescence techniques to locate IL-8 in cryosections of gastric and duodenal biopsies and resected gastric tumour tissue samples, it was found in the epithelium of histologically normal gastric mucosa, with particularly strong expression in the surface cells. Gastric epithelial IL-8 expression was increased in chronic *H. pylori*-associated gastritis, and expression of IL-8 within the lumina propria was evident. Gastric carcinoma cells also expressed IL-8 (Crabtree *et al.*, 1994).

4.2.2 *Experimental systems*

Molecular lesions, cell changes and other precancerous markers have not been measured directly in experimental animals, but *Helicobacter*-induced changes have been mimicked and the effects measured. Thus, Tsujii *et al.* (1993) administered ammonia to rats in the drinking-water for three days a week for one, two, four and eight weeks at a concentration (0.01%) that was considered to be equivalent to that of gastric juice in *H. pylori*-infected people (reported to be 0.015%, as compared with < 0.005% in uninfected people) (Triebling *et al.*, 1991; Neithercut *et al.*, 1993). Controls were given tap-water alone. After four to eight weeks, the mucosal thickness of the antrum but not of the body of the stomach was decreased. Epithelial cell migration rates, measured by incorporation of 5-bromo-2′-deoxyuridine (BrdU), were significantly increased, particularly in the antrum. The BrdU-labelling index was also significantly increased in all ammonia-treated groups. The proliferative zone in the antrum was significantly enlarged as mucosal atrophy developed, whereas in the corpus mucosa enlargement of the proliferative zone occurred despite the absence of mucosal atrophy.

In an investigation by the same group of the possible role of ammonia as a promoter, 85 male Sprague-Dawley rats, five weeks of age, received MNNG at 83 mg/L in the drinking-water for 24 weeks. Forty treated animals were then given tap-water, and 40 were given 0.01% ammonia in the drinking-water. Animals were kept for a further 24 weeks. All rats were killed when moribund or at 48 weeks after the commencement of MNNG treatment. A significantly higher proportion of the rats given MNNG followed by ammonia developed gastric adenocarcinomas (26/37) than those given MNNG followed by tap-water (12/39; $p < 0.01$) (Tsujii *et al.*, 1992).

Administration into the stomachs of mice of a sonicated sample of a cytotoxin-producing strain of *H. pylori* induced epithelial vacuolation and limited infiltration of mononuclear cells into the lamina propra. A sonicated sample of a non-toxin-producing strain did not cause epithelial lesions. The *H. pylori* cytotoxin gene has been cloned into *E. coli*, where a protein was synthesized as a 140-kDa precursor that is processed to a 94-kDa fully active toxin. Oral administration of this recombinant toxin to the mice induced vacuolation but not cell infiltration (Telford *et al.*, 1994).

Consistent with the observation of changes in the location of gastritis with reduced gastric acidity, *H. felis*, which is normally restricted to the antrum, appears in the body of the stomach of rodents given the acid-suppressive drug, omeprazole. Groups of specific pathogen-free BALB/c mice were colonized with *H. felis* and given omeprazole or no treatment for one month; one month after cessation of treatment, *H. felis* was seen in all areas of the stomach in the omeprazole-treated group but only in the cardia and antrum in the controls (Danon *et al.*, 1994). A similar result was obtained in omeprazole-treated *H. felis*-infected rats (Mellgard *et al.*, 1994). [The Working Group noted the incomplete reporting of the data.]

A recently identified bacterium, *H. hepaticus*, was first isolated in association with hepatocellular tumours in mice. Mice infected with this bacterium developed liver lesions, but tumour development has not yet been seen because of the short duration of the experiments reported (Ward *et al.*, 1994).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Helicobacter are spiral, flagellated, gram-negative bacteria that colonize the gastrointestinal tract of human beings and animals. *H. pylori* is restricted to human gastric mucosa and can infect some other primates. *H. pylori* strains are genetically heterogeneous, and this attribute is useful in studies of transmission. *H. pylori* can be cultured, is sensitive to most antibiotics *in vitro* and is characterized by very strong urease activity.

Colonization of the gastric mucosa and subsequent development of gastritis are dependent on bacterial factors, including motility, potent urease activity and specific adherence to gastric epithelium.

H. pylori can be detected in gastric biopsy specimens and indirectly by serology and analysis of breath after ingestion of labelled urea. Standard histological and bacteriological techniques, the polymerase chain reaction and indirect tests are highly sensitive. The rapid urease test on biopsy specimens is practical but less sensitive. Epidemiological studies currently involve use of serological tests and mainly commercially available enzyme-linked immunosorbent assay kits.

H. pylori occurs worldwide and causes a chronic infection which rarely resolves spontaneously. Its prevalence is highest in developing countries and increases rapidly during the first two decades of life, such that 80–90% of the population may be infected by early adulthood. In most developed countries, the prevalence of infection is substantially lower at all ages, and especially in childhood. The prevalence increases gradually throughout life up to the age of 70–80 years. The prevalence in both developed and developing countries is higher among people in lower socioeconomic classes and may be associated with crowding in childhood. A progressive reduction in the rate of infection early in life of people in successive birth cohorts has been observed in developed countries. Transmission occurs from one person to another; both oral–oral and oral–faecal routes have been postulated.

H. pylori causes gastritis in all infected people. This is accompanied by a specific, systemic immunoglobulin G response. Nevertheless, many such infections are asymptomatic. In some people, the infection gives rise to duodenal or gastric ulceration. The infection can be eradicated successfuly with several regimens in which different drugs are combined. Eradication of *H. pylori* resolves gastritis, prevents recurrence of peptic ulcer disease and leads to a significant decline in immunoglobulin response within six months.

5.2 Human carcinogenicity data

Six studies in which estimates of prevalence of infection by *H. pylori* were related to estimates of concurrent or earlier incidence of or mortality from cancer of the stomach in five or fewer populations show no consistent association between these variables. Significantly positive geographical correlations were observed, however, in two larger studies in which the ranges of cancer incidence and mortality were much wider: one in 46 rural populations in China and the other in 17 populations in Europe, Japan and the USA. The populations of

certain developing countries, including many in Africa and some in Asia, have low rates of gastric cancer; the prevalence of *H. pylori* infection has been studied in some of these populations and is known to be high.

The association between prior seropositivity for *H. pylori* and subsequent gastric cancer has been evaluated in three cohort studies, yielding 29–109 cases of gastric cancer. Significant positive associations were observed in all three, with estimated relative risks, based on case–control analyses within the cohorts, varying from 2.8 to 6.0. In a pooled analysis of the three studies, the relative risk was 3.8, which was significant, and there was a significant trend towards increasing estimated relative risks with increasing length of follow-up. In these cohort studies, potential confounding by dietary and other factors that have previously been associated with gastric cancer was not assessed. The extent to which such factors could have contributed to the association between gastric cancer and infection with *H. pylori* is difficult to estimate in view of the imprecision of assessments of past dietary habits.

Nine retrospective case–control studies have addressed the association between seroprevalence for *H. pylori* infection and incidence of gastric cancer. The estimated relative risks for gastric cancer were elevated in six studies, ranging from 1.2 to 4.2, and were significant in three studies. In a number of studies, the control series may not have been representative of the population that gave rise to the cases, either because of the method of sampling (e.g. subjects requiring gastrointestinal investigation) or because of exclusions on the basis of a history of gastric symptoms or disease.

When appropriate stratifications of the results of the prospective and retrospective studies were reported, the association between infection with *H. pylori* and gastric cancer was stronger in younger patients and for cancers at sites other than the cardia. The association was similarly strong for the intestinal and diffuse histological types of cancer.

The association between *H. pylori* infection and gastric lymphoma has been investigated in some studies. In two series of 110 and 178 patients with gastric B-cell mucosa-associated lymphoid tissue lymphomas, 92 and 98%, respectively, had histological evidence of *H. pylori* infection. In two studies of treatment, five of six patients and 12 of 16 patients showed tumour regression after therapy to eradicate *H. pylori*. Thirty-three cases of gastric non-Hodgkin's lymphoma were observed in a cohort study of patients with *H. pylori* infection in the USA and Norway, giving a significant estimated relative risk of 6.3.

5.3 Animal carcinogenicity data

No adequate study on *H. pylori* was available.

5.4 Other relevant data

The gastric precancerous process is characterized by sequential lesions of the gastric mucosa, namely chronic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia. This constellation of lesions occurs in one major type of gastric adenocarcinoma, the intestinal type, the prevalence of which has been declining in developed countries. The other major type is diffuse carcinoma, which is becoming relatively more frequent in those countries and is associated with chronic nonatrophic gastritis.

H. pylori is the main cause of most types of chronic gastritis. This statement is supported by the observation that gastritis developed after voluntary ingestion of bacterial cultures, the consistent association between infection with the bacterium and gastritis throughout the world and the disappearance of gastritis after successful treatment of the infection.

Three independent cohort studies have shown the progression of gastritis from the non-atrophic to the atrophic form. Epidemiological studies of atrophic gastritis have also shown an association with dietary factors, especially excessive salt intake and inadequate consumption of fresh fruits and vegetables.

The bacteria are present in the human gastric stomach as extracellular colonies in the gastric mucus. In most patients, some bacteria adhere to the epithelial cells. Atrophic gastritis induced by *H. pylori* results in overgrowth of other bacteria.

Several *Helicobacter* species induce gastritis in many domestic and experimental animals. Infection with *H. felis* induced chronic gastritis followed by atrophy in mice.

The mechanisms by which *H. pylori* may increase the risk for gastric cancer are unknown. The bacterium has been shown to increase cell replication in the gastric mucosa. Some strains of *H. pylori* which induce inflammation of the gastric mucosa produce cytotoxin. Cytotoxin-associated strains are predominant in both gastric cancer patients and patients with both duodenal ulcer and atrophic gastritis. A protein associated with cytotoxin-positive *H. pylori* strains (cagA) induces expression of interleukin 8 in gastric mucosa, which appears to be correlated with degree of inflammation.

5.5 Evaluation[1]

There is *sufficient evidence* in humans for the carcinogenicity of infection with *Helicobacter pylori*.

There is *inadequate evidence* in experimental animals for the carcinogenicity of infection with *Helicobacter pylori*.

Overall evaluation[2]

Infection with *Helicobacter pylori* is carcinogenic to humans (Group 1).

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[1]For definition of the italicized terms, see Preamble, pp. 30–34.
[2]Dr T. Shirai disassociated himself from the overall evaluation.

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SUMMARY OF FINAL EVALUATIONS

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
Schistosoma haematobium (infection with)	S	L	1
Schistosoma japonicum (infection with)	L	L	2B
Schistosoma mansoni (infection with)	I	L	3
Opisthorchis viverrini (infection with)	S	L	1
Opisthorchis felineus (infection with)	I	I[a]	3
Clonorchis sinensis (infection with)	L	L	2A[b]
Helicobacter pylori (infection with)	S	I[a]	1

S, sufficient evidence; L, limited evidence; I, inadequate evidence; for definitions of criteria for degrees of evidence and groups, see preamble, pp. 30–34

[a]No data available

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

CUMULATIVE CROSS INDEX TO *IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS*

The volume, page and year of publication are given. References to corrigenda are given in parentheses.

A

A-α-C — *40*, 245 (1986); *Suppl. 7*, 56 (1987)
Acetaldehyde — *36*, 101 (1985) (*corr. 42*, 263); *Suppl. 7*, 77 (1987)

Acetaldehyde formylmethylhydrazone (*see* Gyromitrin)
Acetamide — *7*, 197 (1974); *Suppl. 7*, 389 (1987)
Acetaminophen (*see* Paracetamol)
Acridine orange — *16*, 145 (1978); *Suppl. 7*, 56 (1987)
Acriflavinium chloride — *13*, 31 (1977); *Suppl. 7, 56* (1987)
Acrolein — *19*, 479 (1979); *36*,133 (1985); *Suppl. 7*, 78 (1987)

Acrylamide — *39*, 41 (1986); *Suppl. 7*, 56 (1987); *60*, 389 (1994)

Acrylic acid — *19*, 47 (1979); *Suppl. 7*, 56 (1987)
Acrylic fibres — *19*, 86 (1979); *Suppl. 7*, 56 (1987)
Acrylonitrile — *19*, 73 (1979); *Suppl. 7*, 79 (1987)
Acrylonitrile–butadiene–styrene copolymers — *19*, 91 (1979); *Suppl. 7*, 56 (1987)
Actinolite (*see* Asbestos)
Actinomycins — *10*, 29 (1976) (*corr. 42*, 255); *Suppl. 7*, 80 (1987)

Adriamycin — *10*, 43 (1976); *Suppl. 7*, 82 (1987)
AF-2 — *31*, 47 (1983); *Suppl. 7*, 56 (1987)
Aflatoxins — *1*, 145 (1972) (*corr. 42*, 251); *10*, 51 (1976); *Suppl. 7*, 83 (1987); *56*, 245 (1993)

Aflatoxin B_1 (*see* Aflatoxins)
Aflatoxin B_2 (*see* Aflatoxins)
Aflatoxin G_1 (*see* Aflatoxins)
Aflatoxin G_2 (*see* Aflatoxins)
Aflatoxin M_1 (*see* Aflatoxins)
Agaritine — *31*, 63 (1983); *Suppl. 7*, 56 (1987)
Alcohol drinking — *44* (1988)
Aldicarb — *53*, 93 (1991)
Aldrin — *5*, 25 (1974); *Suppl. 7*, 88 (1987)
Allyl chloride — *36*, 39 (1985); *Suppl. 7*, 56 (1987)
Allyl isothiocyanate — *36*, 55 (1985); *Suppl. 7*, 56 (1987)
Allyl isovalerate — *36*, 69 (1985); *Suppl. 7*, 56 (1987)

Aluminium production	*34*, 37 (1984); *Suppl. 7*, 89 (1987)
Amaranth	*8*, 41 (1975); *Suppl. 7*, 56 (1987)
5-Aminoacenaphthene	*16*, 243 (1978); *Suppl. 7*, 56 (1987)
2-Aminoanthraquinone	*27*, 191 (1982); *Suppl. 7*, 56 (1987)
para-Aminoazobenzene	*8*, 53 (1975); *Suppl. 7*, 390 (1987)
ortho-Aminoazotoluene	*8*, 61 (1975) (*corr. 42*, 254); *Suppl. 7*, 56 (1987)
para-Aminobenzoic acid	*16*, 249 (1978); *Suppl. 7*, 56 (1987)
4-Aminobiphenyl	*1*, 74 (1972) (*corr. 42*, 251); *Suppl. 7*, 91 (1987)
2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (*see* MeIQ)	
2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (*see* MeIQx)	
3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (*see* Trp-P-1)	
2-Aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole (*see* Glu-P-2)	
1-Amino-2-methylanthraquinone	*27*, 199 (1982); *Suppl. 7*, 57 (1987)
2-Amino-3-methylimidazo[4,5-*f*]quinoline (*see* IQ)	
2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (*see* Glu-P-1)	
2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (*see* PhIP)	
2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (*see* MeA-α-C)	
3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (*see* Trp-P-2)	
2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole	*7*, 143 (1974); *Suppl. 7*, 57 (1987)
4-Amino-2-nitrophenol	*16*, 43 (1978); *Suppl. 7*, 57 (1987)
2-Amino-4-nitrophenol	*57*, 167 (1993)
2-Amino-5-nitrophenol	*57*, 177 (1993)
2-Amino-5-nitrothiazole	*31*, 71 (1983); *Suppl. 7*, 57 (1987)
2-Amino-9*H*-pyrido[2,3-*b*]indole (*see* A-α-C)	
11-Aminoundecanoic acid	*39*, 239 (1986); *Suppl. 7*, 57 (1987)
Amitrole	*7*, 31 (1974); *41*, 293 (1986) (*corr.* *52*, 513; *Suppl. 7*, 92 (1987)
Ammonium potassium selenide (*see* Selenium and selenium compounds)	
Amorphous silica (*see also* Silica)	*42*, 39 (1987); *Suppl. 7*, 341 (1987)
Amosite (*see* Asbestos)	
Ampicillin	*50*, 153 (1990)
Anabolic steroids (*see* Androgenic (anabolic) steroids)	
Anaesthetics, volatile	*11*, 285 (1976); *Suppl. 7*, 93 (1987)
Analgesic mixtures containing phenacetin (*see also* Phenacetin)	*Suppl. 7*, 310 (1987)
Androgenic (anabolic) steroids	*Suppl. 7*, 96 (1987)
Angelicin and some synthetic derivatives (*see also* Angelicins)	*40*, 291 (1986)
Angelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives)	*Suppl. 7*, 57 (1987)
Angelicins	*Suppl. 7*, 57 (1987)
Aniline	*4*, 27 (1974) (*corr. 42*, 252); *27*, 39 (1982); *Suppl. 7*, 99 (1987)
ortho-Anisidine	*27*, 63 (1982); *Suppl. 7*, 57 (1987)
para-Anisidine	*27*, 65 (1982); *Suppl. 7*, 57 (1987)
Anthanthrene	*32*, 95 (1983); *Suppl. 7*, 57 (1987)
Anthophyllite (*see* Asbestos)	
Anthracene	*32*, 105 (1983); *Suppl. 7*, 57 (1987)
Anthranilic acid	*16*, 265 (1978); *Suppl. 7*, 57 (1987)
Antimony trioxide	*47*, 291 (1989)
Antimony trisulfide	*47*, 291 (1989)
ANTU (*see* 1-Naphthylthiourea)	

Apholate *9*, 31 (1975); *Suppl. 7*, 57 (1987)
Aramite® *5*, 39 (1974); *Suppl. 7*, 57 (1987)
Areca nut (*see* Betel quid)
Arsanilic acid (*see* Arsenic and arsenic compounds)
Arsenic and arsenic compounds *1*, 41 (1972); *2*, 48 (1973); *23*, 39 (1980); *Suppl. 7*, 100 (1987)
Arsenic pentoxide (*see* Arsenic and arsenic compounds)
Arsenic sulfide (*see* Arsenic and arsenic compounds)
Arsenic trioxide (*see* Arsenic and arsenic compounds)
Arsine (*see* Arsenic and arsenic compounds)
Asbestos *2*, 17 (1973) (*corr. 42*, 252); *14* (1977) (*corr. 42*, 256); Suppl. 7, 106 (1987) (*corr. 45*, 283)
Atrazine *53*, 441 (1991)
Attapulgite *42*, 159 (1987); *Suppl. 7*, 117 (1987)
Auramine (technical-grade) *1*, 69 (1972) (*corr. 42*, 251); *Suppl. 7*, 118 (1987)
Auramine, manufacture of (*see also* Auramine, technical-grade) *Suppl. 7*, 118 (1987)
Aurothioglucose *13*, 39 (1977); *Suppl. 7*, 57 (1987)
Azacitidine *26*, 37 (1981); *Suppl. 7*, 57 (1987); *50*, 47 (1990)
5-Azacytidine (*see* Azacitidine)
Azaserine *10*, 73 (1976) (*corr. 42*, 255); *Suppl. 7*, 57 (1987)
Azathioprine *26*, 47 (1981); *Suppl. 7*, 119 (1987)
Aziridine *9*, 37 (1975); *Suppl. 7*, 58 (1987)
2-(1-Aziridinyl)ethanol *9*, 47 (1975); *Suppl. 7*, 58 (1987)
Aziridyl benzoquinone *9*, 51 (1975); *Suppl. 7*, 58 (1987)
Azobenzene *8*, 75 (1975); *Suppl. 7*, 58 (1987)

B

Barium chromate (*see* Chromium and chromium compounds)
Basic chromic sulfate (*see* Chromium and chromium compounds)
BCNU (*see* Bischloroethyl nitrosourea)
Benz[*a*]acridine *32*, 123 (1983); *Suppl. 7*, 58 (1987)
Benz[*c*]acridine *3*, 241 (1973); *32*, 129 (1983); *Suppl. 7*, 58 (1987)
Benzal chloride (*see also* α-Chlorinated toluenes) *29*, 65 (1982); *Suppl. 7*, 148 (1987)
Benz[*a*]anthracene *3*, 45 (1973); *32*, 135 (1983); *Suppl. 7*, 58 (1987)
Benzene *7*, 203 (1974) (*corr. 42*, 254); *29*, 93, 391 (1982); *Suppl. 7*, 120 (1987)
Benzidine *1*, 80 (1972); *29*, 149, 391 (1982); *Suppl. 7*, 123 (1987)
Benzidine-based dyes *Suppl. 7*, 125 (1987)
Benzo[*b*]fluoranthene *3*, 69 (1973); *32*, 147 (1983); *Suppl. 7*, 58 (1987)
Benzo[*j*]fluoranthene *3*, 82 (1973); *32*, 155 (1983); *Suppl. 7*, 58 (1987)
Benzo[*k*]fluoranthene *32*, 163 (1983); *Suppl. 7*, 58 (1987)
Benzo[*ghi*]fluoranthene *32*, 171 (1983); *Suppl. 7*, 58 (1987)

Benzo[*a*]fluorene	*32*, 177 (1983); *Suppl. 7*, 58 (1987)
Benzo[*b*]fluorene	*32*, 183 (1983); *Suppl. 7*, 58 (1987)
Benzo[*c*]fluorene	*32*, 189 (1983); *Suppl. 7*, 58 (1987)
Benzo[*ghi*]perylene	*32*, 195 (1983); *Suppl. 7*, 58 (1987)
Benzo[*c*]phenanthrene	*32*, 205 (1983); *Suppl. 7*, 58 (1987)
Benzo[*a*]pyrene	*3*, 91 (1973); *32*, 211 (1983); *Suppl. 7*, 58 (1987)
Benzo[*e*]pyrene	*3*, 137 (1973); *32*, 225 (1983); *Suppl. 7*, 58 (1987)
para-Benzoquinone dioxime	*29*, 185 (1982); *Suppl. 7*, 58 (1987)
Benzotrichloride (*see also* α-Chlorinated toluenes)	*29*, 73 (1982); *Suppl. 7*, 148 (1987)
Benzoyl chloride	*29*, 83 (1982) (*corr. 42*, 261); *Suppl. 7*, 126 (1987)
Benzoyl peroxide	*36*, 267 (1985); *Suppl. 7*, 58 (1987)
Benzyl acetate	*40*, 109 (1986); *Suppl. 7*, 58 (1987)
Benzyl chloride (*see also* α-Chlorinated toluenes)	*11*, 217 (1976) (*corr. 42*, 256); *29*, 49 (1982); *Suppl. 7*, 148 (1987)
Benzyl violet 4B	*16*, 153 (1978); *Suppl. 7*, 58 (1987)
Bertrandite (*see* Beryllium and beryllium compounds)	
Beryllium and beryllium compounds	*1*, 17 (1972); *23*, 143 (1980) (*corr. 42*, 260); *Suppl. 7*, 127 (1987); *58*, 41 (1993)
Beryllium acetate (*see* Beryllium and beryllium compounds)	
Beryllium acetate, basic (*see* Beryllium and beryllium compounds)	
Beryllium–aluminium alloy (*see* Beryllium and beryllium compounds)	
Beryllium carbonate (*see* Beryllium and beryllium compounds)	
Beryllium chloride (*see* Beryllium and beryllium compounds)	
Beryllium–copper alloy (*see* Beryllium and beryllium compounds)	
Beryllium–copper–cobalt alloy (*see* Beryllium and beryllium compounds)	
Beryllium fluoride (*see* Beryllium and beryllium compounds)	
Beryllium hydroxide (*see* Beryllium and beryllium compounds)	
Beryllium–nickel alloy (*see* Beryllium and beryllium compounds)	
Beryllium oxide (*see* Beryllium and beryllium compounds)	
Beryllium phosphate (*see* Beryllium and beryllium compounds)	
Beryllium silicate (*see* Beryllium and beryllium compounds)	
Beryllium sulfate (*see* Beryllium and beryllium compounds)	
Beryl ore (*see* Beryllium and beryllium compounds)	
Betel quid	*37*, 141 (1985); *Suppl. 7*, 128 (1987)
Betel-quid chewing (*see* Betel quid)	
BHA (*see* Butylated hydroxyanisole)	
BHT (*see* Butylated hydroxytoluene)	
Bis(1-aziridinyl)morpholinophosphine sulfide	*9*, 55 (1975); *Suppl. 7*, 58 (1987)
Bis(2-chloroethyl)ether	*9*, 117 (1975); *Suppl. 7*, 58 (1987)
N,N-Bis(2-chloroethyl)-2-naphthylamine	*4*, 119 (1974) (*corr. 42*, 253); *Suppl. 7*, 130 (1987)
Bischloroethyl nitrosourea (*see also* Chloroethyl nitrosoureas)	*26*, 79 (1981); *Suppl. 7*, 150 (1987)
1,2-Bis(chloromethoxy)ethane	*15*, 31 (1977); *Suppl. 7*, 58 (1987)
1,4-Bis(chloromethoxymethyl)benzene	*15*, 37 (1977); *Suppl. 7*, 58 (1987)
Bis(chloromethyl)ether	*4*, 231 (1974) (*corr. 42*, 253); *Suppl. 7*, 131 (1987)
Bis(2-chloro-1-methylethyl)ether	*41*, 149 (1986); *Suppl. 7*, 59 (1987)
Bis(2,3-epoxycyclopentyl)ether	*47*, 231 (1989)

Bisphenol A diglycidyl ether (*see* Glycidyl ethers)
Bisulfites (*see* Sulfur dioxide and some sulfites, bisulfites and metabisulfites)
Bitumens — *35*, 39 (1985); *Suppl. 7*, 133 (1987)
Bleomycins — *26*, 97 (1981); *Suppl. 7*, 134 (1987)
Blue VRS — *16*, 163 (1978); *Suppl. 7*, 59 (1987)
Boot and shoe manufacture and repair — *25*, 249 (1981); *Suppl. 7*, 232 (1987)
Bracken fern — *40*, 47 (1986); *Suppl. 7*, 135 (1987)
Brilliant Blue FCF, disodium salt — *16*, 171 (1978) (*corr. 42*, 257); *Suppl. 7*, 59 (1987)
Bromochloroacetonitrile (*see* Halogenated acetonitriles)
Bromodichloromethane — *52*, 179 (1991)
Bromoethane — *52*, 299 (1991)
Bromoform — *52*, 213 (1991)
1,3-Butadiene — *39*, 155 (1986) (*corr. 42*, 264); *Suppl. 7*, 136 (1987); *54*, 237 (1992)
1,4-Butanediol dimethanesulfonate — *4*, 247 (1974); *Suppl. 7*, 137 (1987)
n-Butyl acrylate — *39*, 67 (1986); *Suppl. 7*, 59 (1987)
Butylated hydroxyanisole — *40*, 123 (1986); *Suppl. 7*, 59 (1987)
Butylated hydroxytoluene — *40*, 161 (1986); *Suppl. 7*, 59 (1987)
Butyl benzyl phthalate — *29*, 193 (1982) (*corr. 42*, 261); *Suppl. 7*, 59 (1987)
β-Butyrolactone — *11*, 225 (1976); *Suppl. 7*, 59 (1987)
γ-Butyrolactone — *11*, 231 (1976); *Suppl. 7*, 59 (1987)

C

Cabinet-making (*see* Furniture and cabinet-making)
Cadmium acetate (*see* Cadmium and cadmium compounds)
Cadmium and cadmium compounds — 2, 74 (1973); *11*, 39 (1976) (*corr. 42*, 255); *Suppl. 7*, 139 (1987); *58*, 119 (1993)
Cadmium chloride (*see* Cadmium and cadmium compounds)
Cadmium oxide (*see* Cadmium and cadmium compounds)
Cadmium sulfate (*see* Cadmium and cadmium compounds)
Cadmium sulfide (*see* Cadmium and cadmium compounds)
Caffeic acid — *56*, 115 (1993)
Caffeine — *51*, 291 (1991)
Calcium arsenate (*see* Arsenic and arsenic compounds)
Calcium chromate (*see* Chromium and chromium compounds)
Calcium cyclamate (*see* Cyclamates)
Calcium saccharin (*see* Saccharin)
Cantharidin — *10*, 79 (1976); *Suppl. 7*, 59 (1987)
Caprolactam — *19*, 115 (1979) (*corr. 42*, 258); *39*, 247 (1986) (*corr. 42*, 264); *Suppl. 7*, 390 (1987)
Captafol — *53*, 353 (1991)
Captan — *30*, 295 (1983); *Suppl. 7*, 59 (1987)
Carbaryl — *12*, 37 (1976); *Suppl. 7*, 59 (1987)
Carbazole — *32*, 239 (1983); *Suppl. 7*, 59 (1987)
3-Carbethoxypsoralen — *40*, 317 (1986); *Suppl. 7*, 59 (1987)
Carbon blacks — *3*, 22 (1973); *33*, 35 (1984); *Suppl. 7*, 142 (1987)

Carbon tetrachloride	*1*, 53 (1972); *20*, 371 (1979); *Suppl. 7*, 143 (1987)
Carmoisine	*8*, 83 (1975); *Suppl. 7*, 59 (1987)
Carpentry and joinery	*25*, 139 (1981); *Suppl. 7*, 378 (1987)
Carrageenan	*10*, 181 (1976) (*corr. 42*, 255); *31*, 79 (1983); *Suppl. 7*, 59 (1987)
Catechol	*15*, 155 (1977); *Suppl. 7*, 59 (1987)
CCNU (*see* 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea)	
Ceramic fibres (*see* Man-made mineral fibres)	
Chemotherapy, combined, including alkylating agents (*see* MOPP and other combined chemotherapy including alkylating agents)	
Chlorambucil	*9*, 125 (1975); *26*, 115 (1981); *Suppl. 7*, 144 (1987)
Chloramphenicol	*10*, 85 (1976); *Suppl. 7*, 145 (1987); *50*, 169 (1990)
Chlordane (*see also* Chlordane/Heptachlor)	*20*, 45 (1979) (*corr. 42*, 258)
Chlordane/Heptachlor	*Suppl. 7*, 146 (1987); *53*, 115 (1991)
Chlordecone	*20*, 67 (1979); *Suppl. 7*, 59 (1987)
Chlordimeform	*30*, 61 (1983); *Suppl. 7*, 59 (1987)
Chlorendic acid	*48*, 45 (1990)
Chlorinated dibenzodioxins (other than TCDD)	*15*, 41 (1977); *Suppl. 7*, 59 (1987)
Chlorinated drinking-water	*52*, 45 (1991)
Chlorinated paraffins	*48*, 55 (1990)
α-Chlorinated toluenes	*Suppl. 7*, 148 (1987)
Chlormadinone acetate (*see also* Progestins; Combined oral contraceptives)	*6*, 149 (1974); *21*, 365 (1979)
Chlornaphazine (*see N,N*-Bis(2-chloroethyl)-2-naphthylamine)	
Chloroacetonitrile (*see* Halogenated acetonitriles)	
para-Chloroaniline	*57*, 305 (1993)
Chlorobenzilate	*5*, 75 (1974); *30*, 73 (1983); *Suppl. 7*, 60 (1987)
Chlorodibromomethane	*52*, 243 (1991)
Chlorodifluoromethane	*41*, 237 (1986) (*corr. 51*, 483); *Suppl. 7*, 149 (1987)
Chloroethane	*52*, 315 (1991)
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (*see also* Chloroethyl nitrosoureas)	*26*, 137 (1981) (*corr. 42*, 260); *Suppl. 7*, 150 (1987)
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (*see also* Chloroethyl nitrosoureas)	*Suppl. 7*, 150 (1987)
Chloroethyl nitrosoureas	*Suppl. 7*, 150 (1987)
Chlorofluoromethane	*41*, 229 (1986); *Suppl. 7*, 60 (1987)
Chloroform	*1*, 61 (1972); *20*, 401 (1979); *Suppl. 7*, 152 (1987)
Chloromethyl methyl ether (technical-grade) (*see also* Bis(chloromethyl)ether)	*4*, 239 (1974); *Suppl. 7*, 131 (1987)
(4-Chloro-2-methylphenoxy)acetic acid (*see* MCPA)	
Chlorophenols	*Suppl. 7*, 154 (1987)
Chlorophenols (occupational exposures to)	*41*, 319 (1986)
Chlorophenoxy herbicides	*Suppl. 7*, 156 (1987)
Chlorophenoxy herbicides (occupational exposures to)	*41*, 357 (1986)
4-Chloro-*ortho*-phenylenediamine	*27*, 81 (1982); *Suppl. 7*, 60 (1987)
4-Chloro-*meta*-phenylenediamine	*27*, 82 (1982); *Suppl. 7*, 60 (1987)

Chloroprene — *19*, 131 (1979); *Suppl. 7*, 160 (1987)
Chloropropham — *12*, 55 (1976); *Suppl. 7*, 60 (1987)
Chloroquine — *13*, 47 (1977); *Suppl. 7*, 60 (1987)
Chlorothalonil — *30*, 319 (1983); *Suppl. 7*, 60 (1987)
para-Chloro-*ortho*-toluidine and its strong acid salts (*see also* Chlordimeform) — *16*, 277 (1978); *30*, 65 (1983); *Suppl. 7*, 60 (1987); *48*, 123 (1990)
Chlorotrianisene (*see also* Nonsteroidal oestrogens) — *21*, 139 (1979)
2-Chloro-1,1,1-trifluoroethane — *41*, 253 (1986); *Suppl. 7*, 60 (1987)
Chlorozotocin — *50*, 65 (1990)
Cholesterol — *10*, 99 (1976); *31*, 95 (1983); *Suppl. 7*, 161 (1987)

Chromic acetate (*see* Chromium and chromium compounds)
Chromic chloride (*see* Chromium and chromium compounds)
Chromic oxide (*see* Chromium and chromium compounds)
Chromic phosphate (*see* Chromium and chromium compounds)
Chromite ore (*see* Chromium and chromium compounds)
Chromium and chromium compounds — *2*, 100 (1973); *23*, 205 (1980); *Suppl. 7*, 165 (1987); *49*, 49 (1990) (*corr. 51*, 483)

Chromium carbonyl (*see* Chromium and chromium compounds)
Chromium potassium sulfate (*see* Chromium and chromium compounds)
Chromium sulfate (*see* Chromium and chromium compounds)
Chromium trioxide (*see* Chromium and chromium compounds)
Chrysazin (*see* Dantron)
Chrysene — *3*, 159 (1973); *32*, 247 (1983); *Suppl. 7*, 60 (1987)

Chrysoidine — *8*, 91 (1975); *Suppl. 7*, 169 (1987)
Chrysotile (*see* Asbestos)
CI Acid Orange 3 — *57*, 121 (1993)
CI Acid Red 114 — *57*, 247 (1993)
CI Basic Red 9 — *57*, 215 (1993)
Ciclosporin — *50*, 77 (1990)
CI Direct Blue 15 — *57*, 235 (1993)
CI Disperse Yellow 3 (*see* Disperse Yellow 3)
Cimetidine — *50*, 235 (1990)
Cinnamyl anthranilate — *16*, 287 (1978); *31*, 133 (1983); *Suppl. 7*, 60 (1987)

CI Pigment Red 3 — *57*, 259 (1993)
CI Pigment Red 53:1 (*see* D&C Red No. 9)
Cisplatin — *26*, 151 (1981); *Suppl. 7*, 170 (1987)
Citrinin — *40*, 67 (1986); *Suppl. 7*, 60 (1987)
Citrus Red No. 2 — *8*, 101 (1975) (*corr. 42*, 254); *Suppl. 7*, 60 (1987)

Clofibrate — *24*, 39 (1980); *Suppl. 7*, 171 (1987)
Clomiphene citrate — *21*, 551 (1979); *Suppl. 7*, 172 (1987)
Clonorchis sinensis (infection with) — *61*, 121 (1994)
Coal gasification — *34*, 65 (1984); *Suppl. 7*, 173 (1987)
Coal-tar pitches (*see also* Coal-tars) — *35*, 83 (1985); *Suppl. 7*, 174 (1987)
Coal-tars — *35*, 83 (1985); *Suppl. 7*, 175 (1987)
Cobalt[III] acetate (*see* Cobalt and cobalt compounds)
Cobalt–aluminium–chromium spinel (*see* Cobalt and cobalt compounds)

Cobalt and cobalt compounds	*52*, 363 (1991)
Cobalt[II] chloride (*see* Cobalt and cobalt compounds)	
Cobalt–chromium alloy (*see* Chromium and chromium compounds)	
Cobalt–chromium–molybdenum alloys (*see* Cobalt and cobalt compounds)	
Cobalt metal powder (*see* Cobalt and cobalt compounds)	
Cobalt naphthenate (*see* Cobalt and cobalt compounds)	
Cobalt[II] oxide (*see* Cobalt and cobalt compounds)	
Cobalt[II,III] oxide (*see* Cobalt and cobalt compounds)	
Cobalt[II] sulfide (*see* Cobalt and cobalt compounds)	
Coffee	*51*, 41 (1991) (*corr. 52*, 513)
Coke production	*34*, 101 (1984); *Suppl. 7*, 176 (1987)
Combined oral contraceptives (*see also* Oestrogens, progestins and combinations)	*Suppl. 7*, 297 (1987)
Conjugated oestrogens (*see also* Steroidal oestrogens)	*21*, 147 (1979)
Contraceptives, oral (*see* Combined oral contraceptives; Sequential oral contraceptives)	
Copper 8-hydroxyquinoline	*15*, 103 (1977); *Suppl. 7*, 61 (1987)
Coronene	*32*, 263 (1983); *Suppl. 7*, 61 (1987)
Coumarin	*10*, 113 (1976); *Suppl. 7*, 61 (1987)
Creosotes (*see also* Coal-tars)	*35*, 83 (1985); *Suppl. 7*, 177 (1987)
meta-Cresidine	*27*, 91 (1982); *Suppl. 7*, 61 (1987)
para-Cresidine	*27*, 92 (1982); *Suppl. 7*, 61 (1987)
Crocidolite (*see* Asbestos)	
Crude oil	*45*, 119 (1989)
Crystalline silica (*see also* Silica)	*42*, 39 (1987); *Suppl. 7*, 341 (1987)
Cycasin	*1*, 157 (1972) (*corr. 42*, 251); *10*, 121 (1976); *Suppl. 7*, 61 (1987)
Cyclamates	*22*, 55 (1980); *Suppl. 7*, 178 (1987)
Cyclamic acid (*see* Cyclamates)	
Cyclochlorotine	*10*, 139 (1976); *Suppl. 7*, 61 (1987)
Cyclohexanone	*47*, 157 (1989)
Cyclohexylamine (*see* Cyclamates)	
Cyclopenta[*cd*]pyrene	*32*, 269 (1983); *Suppl. 7*, 61 (1987)
Cyclopropane (*see* Anaesthetics, volatile)	
Cyclophosphamide	*9*, 135 (1975); *26*, 165 (1981); *Suppl. 7*, 182 (1987)

D

2,4-D (*see also* Chlorophenoxy herbicides; Chlorophenoxy herbicides, occupational exposures to)	*15*, 111 (1977)
Dacarbazine	*26*, 203 (1981); *Suppl. 7*, 184 (1987)
Dantron	*50*, 265 (1990) (*corr. 59*, 257)
D&C Red No. 9	*8*, 107 (1975); *Suppl. 7*, 61 (1987); *57*, 203 (1993)
Dapsone	*24*, 59 (1980); *Suppl. 7*, 185 (1987)
Daunomycin	*10*, 145 (1976); *Suppl. 7*, 61 (1987)
DDD (*see* DDT)	
DDE (*see* DDT)	
DDT	*5*, 83 (1974) (*corr. 42*, 253); *Suppl. 7*, 186 (1987); *53*, 179 (1991)

Decabromodiphenyl oxide — *48*, 73 (1990)
Deltamethrin — *53*, 251 (1991)
Deoxynivalenol (*see* Toxins derived from *Fusarium graminearum, F. culmorum* and *F. crookwellense*)
Diacetylaminoazotoluene — *8*, 113 (1975); *Suppl. 7*, 61 (1987)
N,N'-Diacetylbenzidine — *16*, 293 (1978); *Suppl. 7*, 61 (1987)
Diallate — *12*, 69 (1976); *30*, 235 (1983); *Suppl. 7*, 61 (1987)
2,4-Diaminoanisole — *16*, 51 (1978); *27*, 103 (1982); *Suppl. 7*, 61 (1987)
4,4'-Diaminodiphenyl ether — *16*, 301 (1978); *29*, 203 (1982); *Suppl. 7*, 61 (1987)
1,2-Diamino-4-nitrobenzene — *16*, 63 (1978); *Suppl. 7*, 61 (1987)
1,4-Diamino-2-nitrobenzene — *16*, 73 (1978); *Suppl. 7*, 61 (1987); *57*, 185 (1993)
2,6-Diamino-3-(phenylazo)pyridine (*see* Phenazopyridine hydrochloride)
2,4-Diaminotoluene (*see also* Toluene diisocyanates) — *16*, 83 (1978); *Suppl. 7*, 61 (1987)
2,5-Diaminotoluene (*see also* Toluene diisocyanates) — *16*, 97 (1978); *Suppl. 7*, 61 (1987)
ortho-Dianisidine (*see* 3,3'-Dimethoxybenzidine)
Diazepam — *13*, 57 (1977); *Suppl. 7*, 189 (1987)
Diazomethane — *7*, 223 (1974); *Suppl. 7*, 61 (1987)
Dibenz[*a,h*]acridine — *3*, 247 (1973); *32*, 277 (1983); *Suppl. 7*, 61 (1987)
Dibenz[*a,j*]acridine — *3*, 254 (1973); *32*, 283 (1983); *Suppl. 7*, 61 (1987)
Dibenz[*a,c*]anthracene — *32*, 289 (1983) (*corr. 42*, 262); *Suppl. 7*, 61 (1987)
Dibenz[*a,h*]anthracene — *3*, 178 (1973) (*corr. 43*, 261); *32*, 299 (1983); *Suppl. 7*, 61 (1987)
Dibenz[*a,j*]anthracene — *32*, 309 (1983); *Suppl. 7*, 61 (1987)
7*H*-Dibenzo[*c,g*]carbazole — *3*, 260 (1973); *32*, 315 (1983); *Suppl. 7*, 61 (1987)
Dibenzodioxins, chlorinated (other than TCDD) [*see* Chlorinated dibenzodioxins (other than TCDD)]
Dibenzo[*a,e*]fluoranthene — *32*, 321 (1983); *Suppl. 7*, 61 (1987)
Dibenzo[*h,rst*]pentaphene — *3*, 197 (1973); *Suppl. 7*, 62 (1987)
Dibenzo[*a,e*]pyrene — *3*, 201 (1973); *32*, 327 (1983); *Suppl. 7*, 62 (1987)
Dibenzo[*a,h*]pyrene — *3*, 207 (1973); *32*, 331 (1983); *Suppl. 7*, 62 (1987)
Dibenzo[*a,i*]pyrene — *3*, 215 (1973); *32*, 337 (1983); *Suppl. 7*, 62 (1987)
Dibenzo[*a,l*]pyrene — *3*, 224 (1973); *32*, 343 (1983); *Suppl. 7*, 62 (1987)
Dibromoacetonitrile (*see* Halogenated acetonitriles)
1,2-Dibromo-3-chloropropane — *15*, 139 (1977); *20*, 83 (1979); *Suppl. 7*, 191 (1987)
Dichloroacetonitrile (*see* Halogenated acetonitriles)
Dichloroacetylene — *39*, 369 (1986); *Suppl. 7*, 62 (1987)
ortho-Dichlorobenzene — *7*, 231 (1974); *29*, 213 (1982); *Suppl. 7*, 192 (1987)

para-Dichlorobenzene	*7*, 231 (1974); *29*, 215 (1982); *Suppl. 7*, 192 (1987)
3,3′-Dichlorobenzidine	*4*, 49 (1974); *29*, 239 (1982); *Suppl. 7*, 193 (1987)
trans-1,4-Dichlorobutene	*15*, 149 (1977); *Suppl. 7*, 62 (1987)
3,3′-Dichloro-4,4′-diaminodiphenyl ether	*16*, 309 (1978); *Suppl. 7*, 62 (1987)
1,2-Dichloroethane	*20*, 429 (1979); *Suppl. 7*, 62 (1987)
Dichloromethane	*20*, 449 (1979); *41*, 43 (1986); *Suppl. 7*, 194 (1987)
2,4-Dichlorophenol (*see* Chlorophenols; Chlorophenols, occupational exposures to)	
(2,4-Dichlorophenoxy)acetic acid (*see* 2,4-D)	
2,6-Dichloro-*para*-phenylenediamine	*39*, 325 (1986); *Suppl. 7*, 62 (1987)
1,2-Dichloropropane	*41*, 131 (1986); *Suppl. 7*, 62 (1987)
1,3-Dichloropropene (technical-grade)	*41*, 113 (1986); *Suppl. 7*, 195 (1987)
Dichlorvos	*20*, 97 (1979); *Suppl. 7*, 62 (1987); *53*, 267 (1991)
Dicofol	*30*, 87 (1983); *Suppl. 7*, 62 (1987)
Dicyclohexylamine (*see* Cyclamates)	
Dieldrin	*5*, 125 (1974); *Suppl. 7*, 196 (1987)
Dienoestrol (*see also* Nonsteroidal oestrogens)	*21*, 161 (1979)
Diepoxybutane	*11*, 115 (1976) (*corr. 42*, 255); *Suppl. 7*, 62 (1987)
Diesel and gasoline engine exhausts	*46*, 41 (1989)
Diesel fuels	*45*, 219 (1989) (*corr. 47*, 505)
Diethyl ether (*see* Anaesthetics, volatile)	
Di(2-ethylhexyl)adipate	*29*, 257 (1982); *Suppl. 7*, 62 (1987)
Di(2-ethylhexyl)phthalate	*29*, 269 (1982) (*corr. 42*, 261); *Suppl. 7*, 62 (1987)
1,2-Diethylhydrazine	*4*, 153 (1974); *Suppl. 7*, 62 (1987)
Diethylstilboestrol	*6*, 55 (1974); *21*, 173 (1979) (*corr. 42*, 259); *Suppl. 7*, 273 (1987)
Diethylstilboestrol dipropionate (*see* Diethylstilboestrol)	
Diethyl sulfate	*4*, 277 (1974); *Suppl. 7*, 198 (1987); *54*, 213 (1992)
Diglycidyl resorcinol ether	*11*, 125 (1976); *36*, 181 (1985); *Suppl. 7*, 62 (1987)
Dihydrosafrole	*1*, 170 (1972); *10*, 233 (1976); *Suppl. 7*, 62 (1987)
1,8-Dihydroxyanthraquinone (*see* Dantron)	
Dihydroxybenzenes (*see* Catechol; Hydroquinone; Resorcinol)	
Dihydroxymethylfuratrizine	*24*, 77 (1980); *Suppl. 7*, 62 (1987)
Diisopropyl sulfate	*54*, 229 (1992)
Dimethisterone (*see also* Progestins; Sequential oral contraceptives)	*6*, 167 (1974); *21*, 377 (1979)
Dimethoxane	*15*, 177 (1977); *Suppl. 7*, 62 (1987)
3,3′-Dimethoxybenzidine	*4*, 41 (1974); *Suppl. 7*, 198 (1987)
3,3′-Dimethoxybenzidine-4,4′-diisocyanate	*39*, 279 (1986); *Suppl. 7*, 62 (1987)
para-Dimethylaminoazobenzene	*8*, 125 (1975); *Suppl. 7*, 62 (1987)
para-Dimethylaminoazobenzenediazo sodium sulfonate	*8*, 147 (1975); *Suppl. 7*, 62 (1987)
trans-2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)-vinyl]-1,3,4-oxadiazole	*7*, 147 (1974) (*corr. 42*, 253); *Suppl. 7*, 62 (1987)

4,4′-Dimethylangelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives) — *Suppl. 7*, 57 (1987)
4,5′-Dimethylangelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives) — *Suppl. 7*, 57 (1987)
2,6-Dimethylaniline — *57*, 323 (1993)
N,*N*-Dimethylaniline — *57*, 337 (1993)
Dimethylarsinic acid (*see* Arsenic and arsenic compounds)
3,3′-Dimethylbenzidine — *1*, 87 (1972); *Suppl. 7*, 62 (1987)
Dimethylcarbamoyl chloride — *12*, 77 (1976); *Suppl. 7*, 199 (1987)
Dimethylformamide — *47*, 171 (1989)
1,1-Dimethylhydrazine — *4*, 137 (1974); *Suppl. 7*, 62 (1987)
1,2-Dimethylhydrazine — *4*, 145 (1974) (*corr. 42*, 253); *Suppl. 7*, 62 (1987)
Dimethyl hydrogen phosphite — *48*, 85 (1990)
1,4-Dimethylphenanthrene — *32*, 349 (1983); *Suppl. 7*, 62 (1987)
Dimethyl sulfate — *4*, 271 (1974); *Suppl. 7*, 200 (1987)
3,7-Dinitrofluoranthene — *46*, 189 (1989)
3,9-Dinitrofluoranthene — *46*, 195 (1989)
1,3-Dinitropyrene — *46*, 201 (1989)
1,6-Dinitropyrene — *46*, 215 (1989)
1,8-Dinitropyrene — *33*, 171 (1984); *Suppl. 7*, 63 (1987); *46*, 231 (1989)
Dinitrosopentamethylenetetramine — *11*, 241 (1976); *Suppl. 7*, 63 (1987)
1,4-Dioxane — *11*, 247 (1976); *Suppl. 7*, 201 (1987)
2,4′-Diphenyldiamine — *16*, 313 (1978); *Suppl. 7*, 63 (1987)
Direct Black 38 (*see also* Benzidine-based dyes) — *29*, 295 (1982) (*corr. 42*, 261)
Direct Blue 6 (*see also* Benzidine-based dyes) — *29*, 311 (1982)
Direct Brown 95 (*see also* Benzidine-based dyes) — *29*, 321 (1982)
Disperse Blue 1 — *48*, 139 (1990)
Disperse Yellow 3 — *8*, 97 (1975); *Suppl. 7*, 60 (1987); *48*, 149 (1990)
Disulfiram — *12*, 85 (1976); *Suppl. 7*, 63 (1987)
Dithranol — *13*, 75 (1977); *Suppl. 7*, 63 (1987)
Divinyl ether (*see* Anaesthetics, volatile)
Dulcin — *12*, 97 (1976); *Suppl. 7*, 63 (1987)

E

Endrin — *5*, 157 (1974); *Suppl. 7*, 63 (1987)
Enflurane (*see* Anaesthetics, volatile)
Eosin — *15*, 183 (1977); *Suppl. 7*, 63 (1987)
Epichlorohydrin — *11*, 131 (1976) (*corr. 42*, 256); *Suppl. 7*, 202 (1987)
1,2-Epoxybutane — *47*, 217 (1989)
1-Epoxyethyl-3,4-epoxycyclohexane (*see* 4-Vinylcyclohexene diepoxide)
3,4-Epoxy-6-methylcyclohexylmethyl-3,4-epoxy-6-methylcyclohexane carboxylate — *11*, 147 (1976); *Suppl. 7*, 63 (1987)
cis-9,10-Epoxystearic acid — *11*, 153 (1976); *Suppl. 7*, 63 (1987)
Erionite — *42*, 225 (1987); *Suppl. 7*, 203 (1987)
Ethinyloestradiol (*see also* Steroidal oestrogens) — *6*, 77 (1974); *21*, 233 (1979)
Ethionamide — *13*, 83 (1977); *Suppl. 7*, 63 (1987)

Ethyl acrylate	*19*, 57 (1979); *39*, 81 (1986); *Suppl. 7*, 63 (1987)
Ethylene	*19*, 157 (1979); *Suppl. 7*, 63 (1987); *60*, 45 (1994)
Ethylene dibromide	*15*, 195 (1977); *Suppl. 7*, 204 (1987)
Ethylene oxide	*11*, 157 (1976); *36*, 189 (1985) (*corr. 42*, 263); *Suppl. 7*, 205 (1987); *60*, 73 (1994)
Ethylene sulfide	*11*, 257 (1976); *Suppl. 7*, 63 (1987)
Ethylene thiourea	*7*, 45 (1974); *Suppl. 7*, 207 (1987)
2-Ethylhexyl acrylate	*60*, 475 (1994)
Ethyl methanesulfonate	*7*, 245 (1974); *Suppl. 7*, 63 (1987)
N-Ethyl-*N*-nitrosourea	*1*, 135 (1972); *17*, 191 (1978); *Suppl. 7*, 63 (1987)
Ethyl selenac (*see also* Selenium and selenium compounds)	*12*, 107 (1976); *Suppl. 7*, 63 (1987)
Ethyl tellurac	*12*, 115 (1976); *Suppl. 7*, 63 (1987)
Ethynodiol diacetate (*see also* Progestins; Combined oral contraceptives)	*6*, 173 (1974); *21*, 387 (1979)
Eugenol	*36*, 75 (1985); *Suppl. 7*, 63 (1987)
Evans blue	*8*, 151 (1975); *Suppl. 7*, 63 (1987)

F

Fast Green FCF	*16*, 187 (1978); *Suppl. 7*, 63 (1987)
Fenvalerate	*53*, 309 (1991)
Ferbam	*12*, 121 (1976) (*corr. 42*, 256); *Suppl. 7*, 63 (1987)
Ferric oxide	*1*, 29 (1972); *Suppl. 7*, 216 (1987)
Ferrochromium (*see* Chromium and chromium compounds)	
Fluometuron	*30*, 245 (1983); *Suppl. 7*, 63 (1987)
Fluoranthene	*32*, 355 (1983); *Suppl. 7*, 63 (1987)
Fluorene	*32*, 365 (1983); *Suppl. 7*, 63 (1987)
Fluorescent lighting (exposure to) (*see* Ultraviolet radiation)	
Fluorides (inorganic, used in drinking-water)	*27*, 237 (1982); *Suppl. 7*, 208 (1987)
5-Fluorouracil	*26*, 217 (1981); *Suppl. 7*, 210 (1987)
Fluorspar (*see* Fluorides)	
Fluosilicic acid (*see* Fluorides)	
Fluroxene (*see* Anaesthetics, volatile)	
Formaldehyde	*29*, 345 (1982); *Suppl. 7*, 211 (1987)
2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole	*7*, 151 (1974) (*corr. 42*, 253); *Suppl. 7*, 63 (1987)
Frusemide (*see* Furosemide)	
Fuel oils (heating oils)	*45*, 239 (1989) (*corr. 47*, 505)
Fumonisin B_1 (*see* Toxins derived from *Fusarium moniliforme*)	
Fumonisin B_2 (*see* Toxins derived from *Fusarium moniliforme*)	
Furazolidone	*31*, 141 (1983); *Suppl. 7*, 63 (1987)
Furniture and cabinet-making	*25*, 99 (1981); *Suppl. 7*, 380 (1987)
Furosemide	*50*, 277 (1990)
2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (*see* AF-2)	
Fusarenon-X (*see* Toxins derived from *Fusarium graminearum*, *F. culmorum* and *F. crookwellense*)	

Fusarenone-X (*see* Toxins derived from *Fusarium graminearum, F. culmorum* and *F. crookwellense*)
Fusarin C (see Toxins derived from *Fusarium moniliforme)*

G

Gasoline — *45*, 159 (1989) (*corr. 47*, 505)
Gasoline engine exhaust (*see* Diesel and gasoline engine exhausts)
Glass fibres (*see* Man-made mineral fibres)
Glass manufacturing industry, occupational exposures in — *58*, 347 (1993)
Glasswool (*see* Man-made mineral fibres)
Glass filaments (*see* Man-made mineral fibres)
Glu-P-1 — *40*, 223 (1986); *Suppl. 7*, 64 (1987)
Glu-P-2 — *40*, 235 (1986); *Suppl. 7*, 64 (1987)
L-Glutamic acid, 5-[2-(4-hydroxymethyl)phenylhydrazide] (*see* Agaritine)
Glycidaldehyde — *11*, 175 (1976); *Suppl. 7*, 64 (1987)
Glycidyl ethers — *47*, 237 (1989)
Glycidyl oleate — 11, 183 (1976); *Suppl. 7*, 64 (1987)
Glycidyl stearate — *11*, 187 (1976); *Suppl. 7*, 64 (1987)
Griseofulvin — *10*, 153 (1976); *Suppl. 7*, 391 (1987)
Guinea Green B — *16*, 199 (1978); *Suppl. 7*, 64 (1987)
Gyromitrin — *31*, 163 (1983); *Suppl. 7*, 391 (1987)

H

Haematite — *1*, 29 (1972); *Suppl. 7*, 216 (1987)
Haematite and ferric oxide — *Suppl. 7*, 216 (1987)
Haematite mining, underground, with exposure to radon — *1*, 29 (1972); *Suppl. 7*, 216 (1987)
Hairdressers and barbers (occupational exposure as) — *57*, 43 (1993)
Hair dyes, epidemiology of — *16*, 29 (1978); *27*, 307 (1982); *52*, 269 (1991)
Halogenated acetonitriles
Halothane (*see* Anaesthetics, volatile)
HC Blue No. 1 — *57*, 129 (1993)
HC Blue No. 2 — *57*, 143 (1993)
α-HCH (*see* Hexachlorocyclohexanes)
β-HCH (*see* Hexachlorocyclohexanes)
γ-HCH (*see* Hexachlorocyclohexanes)
HC Red No. 3 — *57*, 153 (1993)
HC Yellow No. 4 — *57*, 159 (1993)
Heating oils (*see* Fuel oils)
Helicobacter pylori (infection with) — *61*, 177 (1994)
Hepatitis B virus — *59*. 45 (1994)
Hepatitis C virus — *59*, 165 (1994)
Hepatitis D virus — *59*, 223 (1994)
Heptachlor (*see also* Chlordane/Heptachlor) — *5*, 173 (1974); *20*, 129 (1979)
Hexachlorobenzene — *20*, 155 (1979); *Suppl. 7*, 219 (1987)
Hexachlorobutadiene — *20*, 179 (1979); *Suppl. 7*, 64 (1987)
Hexachlorocyclohexanes — *5*, 47 (1974); *20*, 195 (1979) (*corr. 42*, 258); *Suppl. 7*, 220 (1987)

Hexachlorocyclohexane, technical-grade (*see* Hexachlorocyclohexanes)

Hexachloroethane	*20*, 467 (1979); *Suppl. 7*, 64 (1987)
Hexachlorophene	*20*, 241 (1979); *Suppl. 7*, 64 (1987)
Hexamethylphosphoramide	*15*, 211 (1977); *Suppl. 7*, 64 (1987)
Hexoestrol (*see* Nonsteroidal oestrogens)	
Hycanthone mesylate	*13*, 91 (1977); *Suppl. 7*, 64 (1987)
Hydralazine	*24*, 85 (1980); *Suppl. 7*, 222 (1987)
Hydrazine	*4*, 127 (1974); *Suppl. 7*, 223 (1987)
Hydrochloric acid	*54*, 189 (1992)
Hydrochlorothiazide	*50*, 293 (1990)
Hydrogen peroxide	*36*, 285 (1985); *Suppl. 7*, 64 (1987)
Hydroquinone	*15*, 155 (1977); *Suppl. 7*, 64 (1987)
4-Hydroxyazobenzene	*8*, 157 (1975); *Suppl. 7*, 64 (1987)
17α-Hydroxyprogesterone caproate (*see also* Progestins)	*21*, 399 (1979) (*corr. 42*, 259)
8-Hydroxyquinoline	*13*, 101 (1977); *Suppl. 7*, 64 (1987)
8-Hydroxysenkirkine	*10*, 265 (1976); *Suppl. 7*, 64 (1987)
Hypochlorite salts	*52*, 159 (1991)

I

Indeno[1,2,3-*cd*]pyrene	*3*, 229 (1973); *32*, 373 (1983); *Suppl. 7*, 64 (1987)
Inorganic acids (*see* Sulfuric acid and other strong inorganic acids, occupational exposures to mists and vapours from)	
Insecticides, occupational exposures in spraying and application of	*53*, 45 (1991)
IQ	*40*, 261 (1986); *Suppl. 7*, 64 (1987); *56*, 165 (1993)
Iron and steel founding	*34*, 133 (1984); *Suppl. 7*, 224 (1987)
Iron-dextran complex	*2*, 161 (1973); *Suppl. 7*, 226 (1987)
Iron-dextrin complex	*2*, 161 (1973) (*corr. 42*, 252); *Suppl. 7*, 64 (1987)
Iron oxide (*see* Ferric oxide)	
Iron oxide, saccharated (*see* Saccharated iron oxide)	
Iron sorbitol–citric acid complex	*2*, 161 (1973); *Suppl. 7*, 64 (1987)
Isatidine	*10*, 269 (1976); *Suppl. 7*, 65 (1987)
Isoflurane (*see* Anaesthetics, volatile)	
Isoniazid (*see* Isonicotinic acid hydrazide)	
Isonicotinic acid hydrazide	*4*, 159 (1974); *Suppl. 7*, 227 (1987)
Isophosphamide	*26*, 237 (1981); *Suppl. 7*, 65 (1987)
Isoprene	*60*, 215 (1994)
Isopropanol	*5*, 223 (1977); *Suppl. 7*, 229 (1987)
Isopropanol manufacture (strong-acid process) (*see also* Isopropyl alcohol; Sulfuric acid and other strong inorganic acids, occupational exposures to mists and vapours from)	*Suppl. 7*, 229 (1987)
Isopropyl oils	*15*, 223 (1977); *Suppl. 7*, 229 (1987)
Isosafrole	*1*, 169 (1972); *10*, 232 (1976); *Suppl. 7*, 65 (1987)

J

Jacobine	*10*, 275 (1976); *Suppl. 7*, 65 (1987)
Jet fuel	*45*, 203 (1989)
Joinery (*see* Carpentry and joinery)	

K

Kaempferol — 31, 171 (1983); *Suppl. 7*, 65 (1987)
Kepone (*see* Chlordecone)

L

Lasiocarpine — *10*, 281 (1976); *Suppl. 7*, 65 (1987)
Lauroyl peroxide — *36*, 315 (1985); Suppl. 7, 65 (1987)
Lead acetate (*see* Lead and lead compounds)
Lead and lead compounds — *1*, 40 (1972) (*corr. 42*, 251); *2*, 52, 150 (1973); *12*, 131 (1976); *23*, 40, 208, 209, 325 (1980); *Suppl. 7*, 230 (1987)
Lead arsenate (*see* Arsenic and arsenic compounds)
Lead carbonate (*see* Lead and lead compounds)
Lead chloride (*see* Lead and lead compounds)
Lead chromate (*see* Chromium and chromium compounds)
Lead chromate oxide (*see* Chromium and chromium compounds)
Lead naphthenate (*see* Lead and lead compounds)
Lead nitrate (*see* Lead and lead compounds)
Lead oxide (*see* Lead and lead compounds)
Lead phosphate (*see* Lead and lead compounds)
Lead subacetate (*see* Lead and lead compounds)
Lead tetroxide (*see* Lead and lead compounds)
Leather goods manufacture — *25*, 279 (1981); *Suppl. 7*, 235 (1987)
Leather industries — *25*, 199 (1981); *Suppl. 7*, 232 (1987)
Leather tanning and processing — *25*, 201 (1981); *Suppl. 7*, 236 (1987)
Ledate (*see also* Lead and lead compounds) — *12*, 131 (1976)
Light Green SF — *16*, 209 (1978); *Suppl. 7*, 65 (1987)
d-Limonene — *56*, 135 (1993)
Lindane (*see* Hexachlorocyclohexanes)
Liver flukes (*see Clonorchis sinensis, Opisthorchis felineus and Opisthorchis viverrini*)
The lumber and sawmill industries (including logging) — *25*, 49 (1981); *Suppl. 7*, 383 (1987)
Luteoskyrin — *10*, 163 (1976); *Suppl. 7*, 65 (1987)
Lynoestrenol (*see also* Progestins; Combined oral contraceptives) — *21*, 407 (1979)

M

Magenta — *4*, 57 (1974) (*corr. 42*, 252); *Suppl. 7*, 238 (1987); *57*, 215 (1993)
Magenta, manufacture of (*see also* Magenta) — *Suppl. 7*, 238 (1987)
Malathion — *30*, 103 (1983); *Suppl. 7*, 65 (1987)
Maleic hydrazide — *4*, 173 (1974) (*corr. 42*, 253); *Suppl. 7*, 65 (1987)
Malonaldehyde — *36*, 163 (1985); *Suppl. 7*, 65 (1987)
Maneb — *12*, 137 (1976); *Suppl. 7*, 65 (1987)
Man-made mineral fibres — *43*, 39 (1988)
Mannomustine — *9*, 157 (1975); *Suppl. 7*, 65 (1987)
Mate — *51*, 273 (1991)
MCPA (*see also* Chlorophenoxy herbicides; Chlorophenoxy herbicides, occupational exposures to) — *30*, 255 (1983)
MeA-α-C — *40*, 253 (1986); *Suppl. 7*, 65 (1987)

Medphalan	*9*, 168 (1975); *Suppl. 7*, 65 (1987)
Medroxyprogesterone acetate	*6*, 157 (1974); *21*, 417 (1979) (*corr. 42*, 259); *Suppl. 7*, 289 (1987)
Megestrol acetate (*see* also Progestins; Combined oral contraceptives)	
MeIQ	*40*, 275 (1986); *Suppl. 7*, 65 (1987); *56*, 197 (1993)
MeIQx	*40*, 283 (1986); *Suppl. 7*, 65 (1987) *56*, 211 (1993)
Melamine	*39*, 333 (1986); *Suppl. 7*, 65 (1987)
Melphalan	*9*, 167 (1975); *Suppl. 7*, 239 (1987)
6-Mercaptopurine	*26*, 249 (1981); *Suppl. 7*, 240 (1987)
Mercuric chloride (*see* Mercury and mercury compounds)	
Mercury and mercury compounds	*58*, 239 (1993)
Merphalan	*9*, 169 (1975); *Suppl. 7*, 65 (1987)
Mestranol (*see also* Steroidal oestrogens)	*6*, 87 (1974); *21*, 257 (1979) (*corr. 42*, 259)
Metabisulfites (*see* Sulfur dioxide and some sulfites, bisulfites and metabisulfites)	
Metallic mercury (*see* Mercury and mercury compounds)	
Methanearsonic acid, disodium salt (*see* Arsenic and arsenic compounds)	
Methanearsonic acid, monosodium salt (*see* Arsenic and arsenic compounds	
Methotrexate	*26*, 267 (1981); *Suppl. 7*, 241 (1987)
Methoxsalen (*see* 8-Methoxypsoralen)	
Methoxychlor	*5*, 193 (1974); *20*, 259 (1979); *Suppl. 7*, 66 (1987)
Methoxyflurane (*see* Anaesthetics, volatile)	
5-Methoxypsoralen	*40*, 327 (1986); *Suppl. 7*, 242 (1987)
8-Methoxypsoralen (*see also* 8-Methoxypsoralen plus ultraviolet radiation)	*24*, 101 (1980)
8-Methoxypsoralen plus ultraviolet radiation	*Suppl. 7*, 243 (1987)
Methyl acrylate	*19*, 52 (1979); *39*, 99 (1986); *Suppl. 7*, 66 (1987)
5-Methylangelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives)	*Suppl. 7*, 57 (1987)
2-Methylaziridine	*9*, 61 (1975); *Suppl. 7*, 66 (1987)
Methylazoxymethanol acetate	*1*, 164 (1972); *10*, 131 (1976); *Suppl. 7*, 66 (1987)
Methyl bromide	*41*, 187 (1986) (*corr. 45*, 283); *Suppl. 7*, 245 (1987)
Methyl carbamate	*12*, 151 (1976); *Suppl. 7*, 66 (1987)
Methyl-CCNU [*see* 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea]	
Methyl chloride	*41*, 161 (1986); *Suppl. 7*, 246 (1987)
1-, 2-, 3-, 4-, 5- and 6-Methylchrysenes	*32*, 379 (1983); *Suppl. 7*, 66 (1987)
N-Methyl-*N*,4-dinitrosoaniline	*1*, 141 (1972); *Suppl. 7*, 66 (1987)
4,4′-Methylene bis(2-chloroaniline)	*4*, 65 (1974) (*corr. 42*, 252); *Suppl. 7*, 246 (1987); *57*, 271 (1993)
4,4′-Methylene bis(*N*,*N*-dimethyl)benzenamine	*27*, 119 (1982); *Suppl. 7*, 66 (1987)
4,4′-Methylene bis(2-methylaniline)	*4*, 73 (1974); *Suppl. 7*, 248 (1987)

4,4′-Methylenedianiline — *4*, 79 (1974) (*corr. 42, 252*); *39*, 347 (1986); *Suppl. 7*, 66 (1987)
4,4′-Methylenediphenyl diisocyanate — *19*, 314 (1979); *Suppl. 7*, 66 (1987)
2-Methylfluoranthene — *32*, 399 (1983); *Suppl. 7*, 66 (1987)
3-Methylfluoranthene — *32*, 399 (1983); *Suppl. 7*, 66 (1987)
Methylglyoxal — *51*, 443 (1991)
Methyl iodide — *15*, 245 (1977); *41*, 213 (1986); *Suppl. 7*, 66 (1987)
Methylmercury chloride (*see* Mercury and mercury compounds)
Methylmercury compounds (*see* Mercury and mercury compounds)
Methyl methacrylate — *19*, 187 (1979); *Suppl. 7*, 66 (1987); *60*, 445 (1994)
Methyl methanesulfonate — *7*, 253 (1974); *Suppl. 7*, 66 (1987)
2-Methyl-1-nitroanthraquinone — *27*, 205 (1982); *Suppl. 7*, 66 (1987)
N-Methyl-*N*′-nitro-*N*-nitrosoguanidine — *4*, 183 (1974); *Suppl. 7*, 248 (1987)
3-Methylnitrosaminopropionaldehyde [*see* 3-(*N*-Nitrosomethylamino)-propionaldehyde]
3-Methylnitrosaminopropionitrile [*see* 3-(*N*-Nitrosomethylamino)-propionitrile]
4-(Methylnitrosamino)-4-(3-pyridyl)-1-butanal [*see* 4-(*N*-Nitrosomethylamino)-4-(3-pyridyl)-1-butanal]
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone [*see* 4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone]
N-Methyl-*N*-nitrosourea — *1*, 125 (1972); *17*, 227 (1978); *Suppl. 7*, 66 (1987)
N-Methyl-*N*-nitrosourethane — *4*, 211 (1974); *Suppl. 7*, 66 (1987)
N-Methylolacrylamide — *60*, 435 (1994)
Methyl parathion — *30*, 131 (1983); *Suppl. 7*, 392 (1987)
1-Methylphenanthrene — *32*, 405 (1983); *Suppl. 7*, 66 (1987)
7-Methylpyrido[3,4-*c*]psoralen — *40*, 349 (1986); *Suppl. 7*, 71 (1987)
Methyl red — *8*, 161 (1975); *Suppl. 7*, 66 (1987)
Methyl selenac (*see also* Selenium and selenium compounds) — *12*, 161 (1976); *Suppl. 7*, 66 (1987)
Methylthiouracil — *7*, 53 (1974); *Suppl. 7*, 66 (1987)
Metronidazole — *13*, 113 (1977); *Suppl. 7*, 250 (1987)
Mineral oils — *3*, 30 (1973); *33*, 87 (1984) (*corr. 42*, 262); *Suppl. 7*, 252 (1987)
Mirex — *5*, 203 (1974); *20*, 283 (1979) (*corr. 42*, 258); *Suppl. 7*, 66 (1987)
Mitomycin C — *10*, 171 (1976); *Suppl. 7*, 67 (1987)
MNNG [*see* *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine]
MOCA [*see* 4,4′-Methylene bis(2-chloroaniline)]
Modacrylic fibres — *19*, 86 (1979); *Suppl. 7*, 67 (1987)
Monocrotaline — *10*, 291 (1976); *Suppl. 7*, 67 (1987)
Monuron — *12*, 167 (1976); *Suppl. 7*, 67 (1987); *53*, 467 (1991)
MOPP and other combined chemotherapy including alkylating agents — *Suppl. 7*, 254 (1987)
Morpholine — *47*, 199 (1989)
5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone — *7*, 161 (1974); *Suppl. 7*, 67 (1987)
Mustard gas — *9*, 181 (1975) (*corr. 42*, 254); *Suppl. 7*, 259 (1987)

Myleran (*see* 1,4-Butanediol dimethanesulfonate)

N

Nafenopin — *24*, 125 (1980); *Suppl. 7*, 67 (1987)
1,5-Naphthalenediamine — *27*, 127 (1982); *Suppl. 7*, 67 (1987)
1,5-Naphthalene diisocyanate — *19*, 311 (1979); *Suppl. 7*, 67 (1987)
1-Naphthylamine — *4*, 87 (1974) (*corr. 42*, 253); *Suppl. 7*, 260 (1987)

2-Naphthylamine — *4*, 97 (1974); *Suppl. 7*, 261 (1987)
1-Naphthylthiourea — *30*, 347 (1983); *Suppl. 7*, 263 (1987)
Nickel acetate (*see* Nickel and nickel compounds)
Nickel ammonium sulfate (*see* Nickel and nickel compounds)
Nickel and nickel compounds — *2*, 126 (1973) (*corr. 42*, 252); *11*, 75 (1976); *Suppl. 7*, 264 (1987) (*corr. 45, 283); 49*, 257 (1990)

Nickel carbonate (*see* Nickel and nickel compounds)
Nickel carbonyl (*see* Nickel and nickel compounds)
Nickel chloride (*see* Nickel and nickel compounds)
Nickel–gallium alloy (*see* Nickel and nickel compounds)
Nickel hydroxide (*see* Nickel and nickel compounds)
Nickelocene (*see* Nickel and nickel compounds)
Nickel oxide (*see* Nickel and nickel compounds)
Nickel subsulfide (*see* Nickel and nickel compounds)
Nickel sulfate (*see* Nickel and nickel compounds)
Niridazole — *13*, 123 (1977); *Suppl. 7*, 67 (1987)
Nithiazide — *31*, 179 (1983); *Suppl. 7*, 67 (1987)
Nitrilotriacetic acid and its salts — *48*, 181 (1990)
5-Nitroacenaphthene — *16*, 319 (1978); *Suppl. 7*, 67 (1987)
5-Nitro-*ortho*-anisidine — *27*, 133 (1982); *Suppl. 7*, 67 (1987)
9-Nitroanthracene — *33*, 179 (1984); *Suppl. 7*, 67 (1987)
7-Nitrobenz[*a*]anthracene — *46*, 247 (1989)
6-Nitrobenzo[*a*]pyrene — *33*, 187 (1984); *Suppl. 7*, 67 (1987); *46*, 255 (1989)

4-Nitrobiphenyl — *4*, 113 (1974); *Suppl. 7*, 67 (1987)
6-Nitrochrysene — *33*, 195 (1984); *Suppl. 7*, 67 (1987); *46*, 267 (1989)

Nitrofen (technical-grade) — *30*, 271 (1983); *Suppl. 7*, 67 (1987)
3-Nitrofluoranthene — *33*, 201 (1984); *Suppl. 7*, 67 (1987)
2-Nitrofluorene — *46*, 277 (1989)
Nitrofural — *7*, 171 (1974); *Suppl. 7*, 67 (1987); *50*, 195 (1990)

5-Nitro-2-furaldehyde semicarbazone (*see* Nitrofural)
Nitrofurantoin — *50*, 211 (1990)
Nitrofurazone (*see* Nitrofural)
1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone — *7*, 181 (1974); *Suppl. 7*, 67 (1987)
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide — *1*, 181 (1972); *7*, 185 (1974); *Suppl. 7*, 67 (1987)

Nitrogen mustard — *9*, 193 (1975); *Suppl. 7*, 269 (1987)
Nitrogen mustard *N*-oxide — *9*, 209 (1975); *Suppl. 7*, 67 (1987)
1-Nitronaphthalene — *46*, 291 (1989)
2-Nitronaphthalene — *46*, 303 (1989)

3-Nitroperylene *46*, 313 (1989)
2-Nitro-*para*-phenylenediamine (*see* 1,4-Diamino-2-nitrobenzene)
2-Nitropropane *29*, 331 (1982); *Suppl. 7*, 67 (1987)
1-Nitropyrene *33*, 209 (1984); *Suppl. 7*, 67 (1987); *46*, 321 (1989)
2-Nitropyrene *46*, 359 (1989)
4-Nitropyrene *46*, 367 (1989)
N-Nitrosatable drugs *24*, 297 (1980) (*corr. 42*, 260)
N-Nitrosatable pesticides *30*, 359 (1983)
N'-Nitrosoanabasine *37*, 225 (1985); *Suppl. 7*, 67 (1987)
N'-Nitrosoanatabine *37*, 233 (1985); *Suppl. 7*, 67 (1987)
N-Nitrosodi-*n*-butylamine *4*, 197 (1974); *17*, 51 (1978); *Suppl. 7*, 67 (1987)
N-Nitrosodiethanolamine *17*, 77 (1978); *Suppl. 7*, 67 (1987)
N-Nitrosodiethylamine *1*, 107 (1972) (*corr. 42*, 251); *17*, 83 (1978) (*corr. 42*, 257); *Suppl. 7*, 67 (1987)
N-Nitrosodimethylamine *1*, 95 (1972); *17*, 125 (1978) (*corr. 42*, 257); *Suppl. 7*, 67 (1987)
N-Nitrosodiphenylamine *27*, 213 (1982); *Suppl. 7*, 67 (1987)
para-Nitrosodiphenylamine *27*, 227 (1982) (*corr. 42*, 261); *Suppl. 7*, 68 (1987)
N-Nitrosodi-*n*-propylamine *17*, 177 (1978); *Suppl. 7*, 68 (1987)
N-Nitroso-*N*-ethylurea (*see N*-Ethyl-*N*-nitrosourea)
N-Nitrosofolic acid *17*, 217 (1978); *Suppl. 7*, 68 (1987)
N-Nitrosoguvacine *37*, 263 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosoguvacoline *37*, 263 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosohydroxyproline *17*, 304 (1978); *Suppl. 7*, 68 (1987)
3-(*N*-Nitrosomethylamino)propionaldehyde *37*, 263 (1985); *Suppl. 7*, 68 (1987)
3-(*N*-Nitrosomethylamino)propionitrile *37*, 263 (1985); *Suppl. 7*, 68 (1987)
4-(*N*-Nitrosomethylamino)-4-(3-pyridyl)-1-butanal *37*, 205 (1985); *Suppl. 7*, 68 (1987)
4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone *37*, 209 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosomethylethylamine *17*, 221 (1978); *Suppl. 7*, 68 (1987)
N-Nitroso-*N*-methylurea (*see N*-Methyl-*N*-nitrosourea)
N-Nitroso-*N*-methylurethane (*see N*-Methyl-*N*-nitrosourethane)
N-Nitrosomethylvinylamine *17*, 257 (1978); *Suppl. 7*, 68 (1987)
N-Nitrosomorpholine *17*, 263 (1978); *Suppl. 7*, 68 (1987)
N'-Nitrosonornicotine *17*, 281 (1978); *37*, 241 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosopiperidine *17*, 287 (1978); *Suppl. 7*, 68 (1987)
N-Nitrosoproline *17*, 303 (1978); *Suppl. 7*, 68 (1987)
N-Nitrosopyrrolidine *17*, 313 (1978); *Suppl. 7*, 68 (1987)
N-Nitrososarcosine *17*, 327 (1978); *Suppl. 7*, 68 (1987)
Nitrosoureas, chloroethyl (*see* Chloroethyl nitrosoureas)
5-Nitro-*ortho*-toluidine *48*, 169 (1990)
Nitrous oxide (*see* Anaesthetics, volatile)
Nitrovin *31*, 185 (1983); *Suppl. 7*, 68 (1987)
Nivalenol (*see* Toxins derived from *Fusarium graminearum*, *F. culmorum* and *F. crookwellense*)
NNA [*see* 4-(*N*-Nitrosomethylamino)-4-(3-pyridyl)-1-butanal]
NNK [*see* 4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone]

Nonsteroidal oestrogens (*see also* Oestrogens, progestins and combinations)	*Suppl. 7*, 272 (1987)
Norethisterone (*see also* Progestins; Combined oral contraceptives)	*6*, 179 (1974); *21*, 461 (1979)
Norethynodrel (*see also* Progestins; Combined oral contraceptives	*6*, 191 (1974); *21*, 461 (1979) (*corr. 42*, 259)
Norgestrel (*see also* Progestins, Combined oral contraceptives)	*6*, 201 (1974); *21*, 479 (1979)
Nylon 6	*19*, 120 (1979); *Suppl. 7*, 68 (1987)

O

Ochratoxin A	*10*, 191 (1976); *31*, 191 (1983) (*corr. 42*, 262); *Suppl. 7*, 271 (1987); *56*, 489 (1993)
Oestradiol-17β (*see also* Steroidal oestrogens)	*6*, 99 (1974); *21*, 279 (1979)
Oestradiol 3-benzoate (*see* Oestradiol-17β)	
Oestradiol dipropionate (*see* Oestradiol-17β)	
Oestradiol mustard	*9*, 217 (1975)
Oestradiol-17β-valerate (*see* Oestradiol-17β)	
Oestriol (*see also* Steroidal oestrogens)	*6*, 117 (1974); *21*, 327 (1979)
Oestrogen–progestin combinations (*see* Oestrogens, progestins and combinations)	
Oestrogen–progestin replacement therapy (*see also* Oestrogens, progestins and combinations)	*Suppl. 7*, 308 (1987)
Oestrogen replacement therapy (*see also* Oestrogens, progestins and combinations)	*Suppl. 7*, 280 (1987)
Oestrogens (*see* Oestrogens, progestins and combinations)	
Oestrogens, conjugated (*see* Conjugated oestrogens)	
Oestrogens, nonsteroidal (*see* Nonsteroidal oestrogens)	
Oestrogens, progestins and combinations	*6* (1974); *21* (1979); *Suppl. 7*, 272 (1987)
Oestrogens, steroidal (*see* Steroidal oestrogens)	
Oestrone (*see also* Steroidal oestrogens)	*6*, 123 (1974); *21*, 343 (1979) (*corr. 42*, 259)
Oestrone benzoate (*see* Oestrone)	
Oil Orange SS	*8*, 165 (1975); *Suppl. 7*, 69 (1987)
Opisthorchis felineus (infection with)	*61*, 121 (1994)
Opisthorchis viverrini (infection with)	*61*, 121 (1994)
Oral contraceptives, combined (*see* Combined oral contraceptives)	
Oral contraceptives, investigational (*see* Combined oral contraceptives)	
Oral contraceptives, sequential (*see* Sequential oral contraceptives)	
Orange I	*8*, 173 (1975); *Suppl. 7*, 69 (1987)
Orange G	*8*, 181 (1975); *Suppl. 7*, 69 (1987)
Organolead compounds (*see also* Lead and lead compounds)	*Suppl. 7*, 230 (1987)
Oxazepam	13, 58 (1977); *Suppl. 7*, 69 (1987)
Oxymetholone [*see also* Androgenic (anabolic) steroids]	*13*, 131 (1977)
Oxyphenbutazone	*13*, 185 (1977); *Suppl. 7*, 69 (1987)

P

Paint manufacture and painting (occupational exposures in)	*47*, 329 (1989)

Panfuran S (*see also* Dihydroxymethylfuratrizine) — *24*, 77 (1980); *Suppl. 7*, 69 (1987)
Paper manufacture (*see* Pulp and paper manufacture)
Paracetamol — *50*, 307 (1990)
Parasorbic acid — *10*, 199 (1976) (*corr. 42*, 255); *Suppl. 7*, 69 (1987)

Parathion — *30*, 153 (1983); *Suppl. 7*, 69 (1987)
Patulin — *10*, 205 (1976); *40*, 83 (1986); *Suppl. 7*, 69 (1987)

Penicillic acid — *10*, 211 (1976); *Suppl. 7*, 69 (1987)
Pentachloroethane — *41*, 99 (1986); *Suppl. 7*, 69 (1987)
Pentachloronitrobenzene (*see* Quintozene)
Pentachlorophenol (*see also* Chlorophenols; Chlorophenols, occupational exposures to) — *20*, 303 (1979); *53*, 371 (1991)
Permethrin — *53*, 329 (1991)
Perylene — *32*, 411 (1983); *Suppl. 7*, 69 (1987)
Petasitenine — *31*, 207 (1983); *Suppl. 7*, 69 (1987)
Petasites japonicus (*see* Pyrrolizidine alkaloids)
Petroleum refining (occupational exposures in) — *45*, 39 (1989)
Some petroleum solvents — *47*, 43 (1989)
Phenacetin — *13*, 141 (1977); *24*, 135 (1980); *Suppl. 7*, 310 (1987)

Phenanthrene — *32*, 419 (1983); *Suppl. 7*, 69 (1987)
Phenazopyridine hydrochloride — *8*, 117 (1975); *24*, 163 (1980) (*corr. 42*, 260); *Suppl. 7*, 312 (1987)

Phenelzine sulfate — *24*, 175 (1980); *Suppl. 7*, 312 (1987)
Phenicarbazide — *12*, 177 (1976); *Suppl. 7*, 70 (1987)
Phenobarbital — *13*, 157 (1977); *Suppl. 7*, 313 (1987)
Phenol — *47*, 263 (1989) (*corr. 50*, 385)
Phenoxyacetic acid herbicides (*see* Chlorophenoxy herbicides)
Phenoxybenzamine hydrochloride — *9*, 223 (1975); *24*, 185 (1980); *Suppl. 7*, 70 (1987)

Phenylbutazone — *13*, 183 (1977); *Suppl. 7*, 316 (1987)
meta-Phenylenediamine — *16*, 111 (1978); *Suppl. 7*, 70 (1987)
para-Phenylenediamine — *16*, 125 (1978); *Suppl. 7*, 70 (1987)
Phenyl glycidyl ether (*see* Glycidyl ethers)
N-Phenyl-2-naphthylamine — *16*, 325 (1978) (*corr. 42*, 257); *Suppl. 7*, 318 (1987)

ortho-Phenylphenol — *30*, 329 (1983); *Suppl. 7*, 70 (1987)
Phenytoin — *13*, 201 (1977); *Suppl. 7*, 319 (1987)
PhIP — *56*, 229 (1993)
Pickled vegetables — *56*, 83 (1993)
Picloram — *53*, 481 (1991)
Piperazine oestrone sulfate (*see* Conjugated oestrogens)
Piperonyl butoxide — *30*, 183 (1983); *Suppl. 7*, 70 (1987)
Pitches, coal-tar (*see* Coal-tar pitches)
Polyacrylic acid — *19*, 62 (1979); *Suppl. 7*, 70 (1987)
Polybrominated biphenyls — *18*, 107 (1978); *41*, 261 (1986); *Suppl. 7*, 321 (1987)

Polychlorinated biphenyls — *7*, 261 (1974); *18*, 43 (1978) (*corr. 42*, 258); *Suppl. 7*, 322 (1987)

Polychlorinated camphenes (*see* Toxaphene)
Polychloroprene — *19*, 141 (1979); *Suppl. 7*, 70 (1987)

Polyethylene	*19*, 164 (1979); *Suppl. 7*, 70 (1987)
Polymethylene polyphenyl isocyanate	*19*, 314 (1979); *Suppl. 7*, 70 (1987)
Polymethyl methacrylate	*19*, 195 (1979); *Suppl. 7*, 70 (1987)
Polyoestradiol phosphate (*see* Oestradiol-17β)	
Polypropylene	*19*, 218 (1979); *Suppl. 7*, 70 (1987)
Polystyrene	*19*, 245 (1979); *Suppl. 7*, 70 (1987)
Polytetrafluoroethylene	*19*, 288 (1979); *Suppl. 7*, 70 (1987)
Polyurethane foams	*19*, 320 (1979); *Suppl. 7*, 70 (1987)
Polyvinyl acetate	*19*, 346 (1979); *Suppl. 7*, 70 (1987)
Polyvinyl alcohol	*19*, 351 (1979); *Suppl. 7*, 70 (1987)
Polyvinyl chloride	*7*, 306 (1974); *19*, 402 (1979); *Suppl. 7*, 70 (1987)
Polyvinyl pyrrolidone	*19*, 463 (1979); *Suppl. 7*, 70 (1987)
Ponceau MX	*8*, 189 (1975); *Suppl. 7*, 70 (1987)
Ponceau 3R	*8*, 199 (1975); *Suppl. 7*, 70 (1987)
Ponceau SX	*8*, 207 (1975); *Suppl. 7*, 70 (1987)
Potassium arsenate (*see* Arsenic and arsenic compounds)	
Potassium arsenite (*see* Arsenic and arsenic compounds)	
Potassium bis(2-hydroxyethyl)dithiocarbamate	*12*, 183 (1976); *Suppl. 7*, 70 (1987)
Potassium bromate	*40*, 207 (1986); *Suppl. 7*, 70 (1987)
Potassium chromate (*see* Chromium and chromium compounds)	
Potassium dichromate (*see* Chromium and chromium compounds)	
Prednimustine	*50*, 115 (1990)
Prednisone	*26*, 293 (1981); *Suppl. 7*, 326 (1987)
Procarbazine hydrochloride	*26*, 311 (1981); *Suppl. 7*, 327 (1987)
Proflavine salts	*24*, 195 (1980); *Suppl. 7*, 70 (1987)
Progesterone (*see also* Progestins; Combined oral contraceptives)	*6*, 135 (1974); *21*, 491 (1979) (*corr. 42*, 259)
Progestins (*see also* Oestrogens, progestins and combinations)	*Suppl. 7*, 289 (1987)
Pronetalol hydrochloride	*13*, 227 (1977) (*corr. 42*, 256); *Suppl. 7*, 70 (1987)
1,3-Propane sultone	*4*, 253 (1974) (*corr. 42*, 253); *Suppl. 7*, 70 (1987)
Propham	*12*, 189 (1976); *Suppl. 7*, 70 (1987)
β-Propiolactone	*4*, 259 (1974) (*corr. 42*, 253); *Suppl. 7*, 70 (1987)
n-Propyl carbamate	*12*, 201 (1976); *Suppl. 7*, 70 (1987)
Propylene	*19*, 213 (1979); *Suppl. 7*, 71 (1987); *60*, 161 (1994)
Propylene oxide	*11*, 191 (1976); *36*, 227 (1985) (*corr. 42*, 263); *Suppl. 7*, 328 (1987); *60*, 181 (1994)
Propylthiouracil	*7*, 67 (1974); *Suppl. 7*, 329 (1987)
Ptaquiloside (*see also* Bracken fern)	*40*, 55 (1986); *Suppl. 7*, 71 (1987)
Pulp and paper manufacture	*25*, 157 (1981); *Suppl. 7*, 385 (1987)
Pyrene	*32*, 431 (1983); *Suppl. 7*, 71 (1987)
Pyrido[3,4-*c*]psoralen	*40*, 349 (1986); *Suppl. 7*, 71 (1987)
Pyrimethamine	*13*, 233 (1977); *Suppl. 7*, 71 (1987)
Pyrrolizidine alkaloids (*see* Hydroxysenkirkine; Isatidine; Jacobine; Lasiocarpine; Monocrotaline; Retrorsine; Riddelliine; Seneciphylline; Senkirkine)	

Q

Quercetin (*see also* Bracken fern) *31*, 213 (1983); *Suppl. 7*, 71 (1987)
para-Quinone *15*, 255 (1977); *Suppl. 7*, 71 (1987)
Quintozene *5*, 211 (1974); *Suppl. 7*, 71 (1987)

R

Radon *43*, 173 (1988) (*corr. 45*, 283)
Reserpine *10*, 217 (1976); *24*, 211 (1980) (*corr. 42*, 260); *Suppl. 7*, 330 (1987)
Resorcinol *15*, 155 (1977); *Suppl. 7*, 71 (1987)
Retrorsine *10*, 303 (1976); *Suppl. 7*, 71 (1987)
Rhodamine B *16*, 221 (1978); *Suppl. 7*, 71 (1987)
Rhodamine 6G *16*, 233 (1978); *Suppl. 7*, 71 (1987)
Riddelliine *10*, 313 (1976); *Suppl. 7*, 71 (1987)
Rifampicin *24*, 243 (1980); *Suppl. 7*, 71 (1987)
Rockwool (*see* Man-made mineral fibres)
The rubber industry *28* (1982) (*corr. 42*, 261); *Suppl. 7*, 332 (1987)
Rugulosin *40*, 99 (1986); *Suppl. 7*, 71 (1987)

S

Saccharated iron oxide *2*, 161 (1973); *Suppl. 7*, 71 (1987)
Saccharin *22*, 111 (1980) (*corr. 42*, 259); *Suppl. 7*, 334 (1987)
Safrole *1*, 169 (1972); *10*, 231 (1976); *Suppl. 7*, 71 (1987)
Salted fish *56*, 41 (1993)
The sawmill industry (including logging) [*see* The lumber and sawmill industry (including logging)]
Scarlet Red *8*, 217 (1975); *Suppl. 7*, 71 (1987)
Schistosoma haematobium (infection with) *61*, 45 (1994)
Schistosoma japonicum (infection with) *61*, 45 (1994)
Schistosoma mansoni (infection with) *61*, 45 (1994)
Selenium and selenium compounds *9*, 245 (1975) (*corr. 42*, 255); *Suppl. 7*, 71 (1987)
Selenium dioxide (*see* Selenium and selenium compounds)
Selenium oxide (*see* Selenium and selenium compounds)
Semicarbazide hydrochloride *12*, 209 (1976) (*corr. 42*, 256); *Suppl. 7*, 71 (1987)
Senecio jacobaea L. (*see* Pyrrolizidine alkaloids)
Senecio longilobus (*see* Pyrrolizidine alkaloids)
Seneciphylline *10*, 319, 335 (1976); *Suppl. 7*, 71 (1987)
Senkirkine *10*, 327 (1976); *31*, 231 (1983); *Suppl. 7*, 71 (1987)
Sepiolite *42*, 175 (1987); *Suppl. 7*, 71 (1987)
Sequential oral contraceptives (*see also* Oestrogens, progestins and combinations) *Suppl. 7*, 296 (1987)
Shale-oils *35*, 161 (1985); *Suppl. 7*, 339 (1987)

Shikimic acid (*see also* Bracken fern)	*40*, 55 (1986); *Suppl. 7*, 71 (1987)
Shoe manufacture and repair (*see* Boot and shoe manufacture and repair)	
Silica (*see also* Amorphous silica; Crystalline silica)	*42*, 39 (1987)
Simazine	*53*, 495 (1991)
Slagwool (*see* Man-made mineral fibres)	
Sodium arsenate (*see* Arsenic and arsenic compounds)	
Sodium arsenite (*see* Arsenic and arsenic compounds)	
Sodium cacodylate (*see* Arsenic and arsenic compounds)	
Sodium chlorite	*52*, 145 (1991)
Sodium chromate (*see* Chromium and chromium compounds)	
Sodium cyclamate (*see* Cyclamates)	
Sodium dichromate (*see* Chromium and chromium compounds)	
Sodium diethyldithiocarbamate	*12*, 217 (1976); *Suppl. 7*, 71 (1987)
Sodium equilin sulfate (*see* Conjugated oestrogens)	
Sodium fluoride (*see* Fluorides)	
Sodium monofluorophosphate (*see* Fluorides)	
Sodium oestrone sulfate (*see* Conjugated oestrogens)	
Sodium *ortho*-phenylphenate (*see also ortho*-Phenylphenol)	*30*, 329 (1983); *Suppl. 7*, 392 (1987)
Sodium saccharin (*see* Saccharin)	
Sodium selenate (*see* Selenium and selenium compounds)	
Sodium selenite (*see* Selenium and selenium compounds)	
Sodium silicofluoride (*see* Fluorides)	
Solar radiation	*55* (1992)
Soots	*3*, 22 (1973); *35*, 219 (1985); *Suppl. 7*, 343 (1987)
Spironolactone	*24*, 259 (1980); *Suppl. 7*, 344 (1987)
Stannous fluoride (*see* Fluorides)	
Steel founding (*see* Iron and steel founding)	
Sterigmatocystin	*1*, 175 (1972); *10*, 245 (1976); *Suppl. 7*, 72 (1987)
Steroidal oestrogens (*see also* Oestrogens, progestins and combinations)	*Suppl. 7*, 280 (1987)
Streptozotocin	*4*, 221 (1974); *17*, 337 (1978); *Suppl. 7*, 72 (1987)
Strobane® (*see* Terpene polychlorinates)	
Strontium chromate (*see* Chromium and chromium compounds)	
Styrene	*19*, 231 (1979) (*corr. 42*, 258); *Suppl. 7*, 345 (1987); *60*, 233 (1994)
Styrene-acrylonitrile copolymers	*19*, 97 (1979); *Suppl. 7*, 72 (1987)
Styrene-butadiene copolymers	*19*, 252 (1979); *Suppl. 7*, 72 (1987)
Styrene-7,8-oxide	*11*, 201 (1976); *19*, 275 (1979); *36*, 245 (1985); *Suppl. 7*, 72 (1987); *60*, 321 (1994)
Succinic anhydride	*15*, 265 (1977); *Suppl. 7*, 72 (1987)
Sudan I	*8*, 225 (1975); *Suppl. 7*, 72 (1987)
Sudan II	*8*, 233 (1975); *Suppl. 7*, 72 (1987)
Sudan III	*8*, 241 (1975); *Suppl. 7*, 72 (1987)
Sudan Brown RR	*8*, 249 (1975); *Suppl. 7*, 72 (1987)
Sudan Red 7B	*8*, 253 (1975); *Suppl. 7*, 72 (1987)
Sulfafurazole	*24*, 275 (1980); *Suppl. 7*, 347 (1987)
Sulfallate	*30*, 283 (1983); *Suppl. 7*, 72 (1987)

Sulfamethoxazole — *24*, 285 (1980); *Suppl. 7*, 348 (1987)
Sulfites (*see* Sulfur dioxide and some sulfites, bisulfites and metabisulfites)
Sulfur dioxide and some sulfites, bisulfites and metabisulfites — *54*, 131 (1992)
Sulfur mustard (*see* Mustard gas)
Sulfuric acid and other strong inorganic acids, occupational exposures to mists and vapours from — *54*, 41 (1992)
Sulfur trioxide — *54*, 121 (1992)
Sulphisoxazole (*see* Sulfafurazole)
Sunset Yellow FCF — *8*, 257 (1975); *Suppl. 7*, 72 (1987)
Symphytine — *31*, 239 (1983); *Suppl. 7*, 72 (1987)

T

2,4,5-T (*see also* Chlorophenoxy herbicides; Chlorophenoxy herbicides, occupational exposures to) — *15*, 273 (1977)
Talc — *42*, 185 (1987); *Suppl. 7*, 349 (1987)
Tannic acid — *10*, 253 (1976) (*corr. 42*, 255); *Suppl. 7*, 72 (1987)
Tannins (*see also* Tannic acid) — *10*, 254 (1976); *Suppl. 7*, 72 (1987)
TCDD (*see* 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin)
TDE (*see* DDT)
Tea — *51*, 207 (1991)
Terpene polychlorinates — *5*, 219 (1974); *Suppl. 7*, 72 (1987)
Testosterone (*see also* Androgenic (anabolic) steroids) — *6*, 209 (1974); *21*, 519 (1979)
Testosterone oenanthate (*see* Testosterone)
Testosterone propionate (*see* Testosterone)
2,2′,5,5′-Tetrachlorobenzidine — *27*, 141 (1982); *Suppl. 7*, 72 (1987)
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin — *15*, 41 (1977); *Suppl. 7*, 350 (1987)
1,1,1,2-Tetrachloroethane — *41*, 87 (1986); *Suppl. 7*, 72 (1987)
1,1,2,2-Tetrachloroethane — *20*, 477 (1979); *Suppl. 7*, 354 (1987)
Tetrachloroethylene — *20*, 491 (1979); *Suppl. 7*, 355 (1987)
2,3,4,6-Tetrachlorophenol (*see* Chlorophenols; Chlorophenols, occupational exposures to)
Tetrachlorvinphos — *30*, 197 (1983); *Suppl. 7*, 72 (1987)
Tetraethyllead (*see* Lead and lead compounds)
Tetrafluoroethylene — *19*, 285 (1979); *Suppl. 7*, 72 (1987)
Tetrakis(hydroxymethyl) phosphonium salts — *48*, 95 (1990)
Tetramethyllead (*see* Lead and lead compounds)
Textile manufacturing industry, exposures in — *48*, 215 (1990) (*corr. 51*, 483)
Theobromine — *51*, 421 (1991)
Theophylline — *51*, 391 (1991)
Thioacetamide — *7*, 77 (1974); *Suppl. 7*, 72 (1987)
4,4′-Thiodianiline — *16*, 343 (1978); *27*, 147 (1982); *Suppl. 7*, 72 (1987)
Thiotepa — *9*, 85 (1975); *Suppl. 7*, 368 (1987); *50*, 123 (1990)
Thiouracil — *7*, 85 (1974); *Suppl. 7*, 72 (1987)
Thiourea — *7*, 95 (1974); *Suppl. 7*, 72 (1987)
Thiram — *12*, 225 (1976); *Suppl. 7*, 72 (1987); *53*, 403 (1991)
Titanium dioxide — *47*, 307 (1989)

Tobacco habits other than smoking (*see* Tobacco products, smokeless)	
Tobacco products, smokeless	*37* (1985) (*corr. 42*, 263; *52*, 513); *Suppl. 7*, 357 (1987)
Tobacco smoke	*38* (1986) (*corr. 42*, 263); *Suppl. 7*, 357 (1987)
Tobacco smoking (*see* Tobacco smoke)	
ortho-Tolidine (*see* 3,3′-Dimethylbenzidine)	
2,4-Toluene diisocyanate (*see also* Toluene diisocyanates)	*19*, 303 (1979); *39*, 287 (1986)
2,6-Toluene diisocyanate (*see also* Toluene diisocyanates)	*19*, 303 (1979); *39*, 289 (1986)
Toluene	*47*, 79 (1989)
Toluene diisocyanates	*39*, 287 (1986) (*corr. 42*, 264); *Suppl. 7*, 72 (1987)
Toluenes, α-chlorinated (*see* α-Chlorinated toluenes)	
ortho-Toluenesulfonamide (*see* Saccharin)	
ortho-Toluidine	*16*, 349 (1978); *27*, 155 (1982); *Suppl. 7*, 362 (1987)
Toxaphene	*20*, 327 (1979); *Suppl. 7*, 72 (1987)
T-2 Toxin (*see* Toxins derived from *Fusarium sporotrichioides*)	
Toxins derived from *Fusarium graminearum*, *F. culmorum* and *F. crookwellense*	*11*, 169 (1976); *31*, 153, 279 (1983); *Suppl. 7*, 64, 74 (1987); *56*, 397 (1993)
Toxins derived from *Fusarium moniliforme*	*56*, 445 (1993)
Toxins derived from *Fusarium sporotrichioides*	*31*, 265 (1983); *Suppl. 7*, 73 (1987); *56*, 467 (1993)
Tremolite (*see* Asbestos)	
Treosulfan	*26*, 341 (1981); *Suppl. 7*, 363 (1987)
Triaziquone [*see* Tris(aziridinyl)-*para*-benzoquinone]	
Trichlorfon	*30*, 207 (1983); *Suppl. 7*, 73 (1987)
Trichlormethine	*9*, 229 (1975); *Suppl. 7*, 73 (1987); *50*, 143 (1990)
Trichloroacetonitrile (*see* Halogenated acetonitriles)	
1,1,1-Trichloroethane	*20*, 515 (1979); *Suppl. 7*, 73 (1987)
1,1,2-Trichloroethane	*20*, 533 (1979); *Suppl. 7*, 73 (1987); *52*, 337 (1991)
Trichloroethylene	*11*, 263 (1976); *20*, 545 (1979); *Suppl. 7*, 364 (1987)
2,4,5-Trichlorophenol (*see also* Chlorophenols; Chlorophenols occupational exposures to)	*20*, 349 (1979)
2,4,6-Trichlorophenol (*see also* Chlorophenols; Chlorophenols, occupational exposures to)	*20*, 349 (1979)
(2,4,5-Trichlorophenoxy)acetic acid (*see* 2,4,5-T)	
Trichlorotriethylamine hydrochloride (*see* Trichlormethine)	
T_2-Trichothecene (*see* Toxins derived from *Fusarium sporotrichioides*)	
Triethylene glycol diglycidyl ether	*11*, 209 (1976); *Suppl. 7*, 73 (1987)
Trifluralin	*53*, 515 (1991)
4,4′,6-Trimethylangelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives)	*Suppl. 7*, 57 (1987)
2,4,5-Trimethylaniline	*27*, 177 (1982); *Suppl. 7*, 73 (1987)
2,4,6-Trimethylaniline	*27*, 178 (1982); *Suppl. 7*, 73 (1987)
4,5′,8-Trimethylpsoralen	*40*, 357 (1986); *Suppl. 7*, 366 (1987)
Trimustine hydrochloride (*see* Trichlormethine)	
Triphenylene	*32*, 447 (1983); *Suppl. 7*, 73 (1987)

Tris(aziridinyl)-*para*-benzoquinone *9*, 67 (1975); *Suppl. 7*, 367 (1987)
Tris(1-aziridinyl)phosphine oxide *9*, 75 (1975); *Suppl. 7*, 73 (1987)
Tris(1-aziridinyl)phosphine sulphide (*see* Thiotepa)
2,4,6-Tris(1-aziridinyl)-*s*-triazine *9*, 95 (1975); *Suppl. 7*, 73 (1987)
Tris(2-chloroethyl) phosphate *48*, 109 (1990)
1,2,3-Tris(chloromethoxy)propane *15*, 301 (1977); *Suppl. 7*, 73 (1987)
Tris(2,3-dibromopropyl)phosphate *20*, 575 (1979); *Suppl. 7*, 369 (1987)
Tris(2-methyl-1-aziridinyl)phosphine oxide *9*, 107 (1975); *Suppl. 7*, 73 (1987)
Trp-P-1 *31*, 247 (1983); *Suppl. 7*, 73 (1987)
Trp-P-2 *31*, 255 (1983); *Suppl. 7*, 73 (1987)
Trypan blue *8*, 267 (1975); *Suppl. 7*, 73 (1987)
Tussilago farfara L. (*see* Pyrrolizidine alkaloids)

U

Ultraviolet radiation *40*, 379 (1986); *55* (1992)
Underground haematite mining with exposure to radon *1*, 29 (1972); *Suppl. 7*, 216 (1987)
Uracil mustard *9*, 235 (1975); *Suppl. 7*, 370 (1987)
Urethane *7*, 111 (1974); *Suppl. 7*, 73 (1987)

V

Vat Yellow 4 *48*, 161 (1990)
Vinblastine sulfate *26*, 349 (1981) (*corr. 42*, 261); *Suppl. 7*, 371 (1987)
Vincristine sulfate *26*, 365 (1981); *Suppl. 7*, 372 (1987)
Vinyl acetate *19*, 341 (1979); *39*, 113 (1986); *Suppl. 7*, 73 (1987)
Vinyl bromide *19*, 367 (1979); *39*, 133 (1986); *Suppl. 7*, 73 (1987)
Vinyl chloride *7*, 291 (1974); *19*, 377 (1979) (*corr. 42*, 258); *Suppl. 7*, 373 (1987)
Vinyl chloride–vinyl acetate copolymers *7*, 311 (1976); *19*, 412 (1979) (*corr. 42*, 258); *Suppl. 7*, 73 (1987)
4-Vinylcyclohexene *11*, 277 (1976); *39*, 181 (1986); *Suppl. 7*, 73 (1987); *60*, 347 (1994)
4-Vinylcyclohexene diepoxide *11*, 141 (1976); *Suppl. 7*, 63 (1987); *60*, 361 (1994)
Vinyl fluoride *39*, 147 (1986); *Suppl. 7*, 73 (1987)
Vinylidene chloride *19*, 439 (1979); *39*, 195 (1986); *Suppl. 7*, 376 (1987)
Vinylidene chloride–vinyl chloride copolymers *19*, 448 (1979) (*corr. 42*, 258); *Suppl. 7*, 73 (1987)
Vinylidene fluoride *39*, 227 (1986); *Suppl. 7*, 73 (1987)
N-Vinyl-2-pyrrolidone *19*, 461 (1979); *Suppl. 7*, 73 (1987)
Vinyl toluene *60*, 373 (1994)

W

Welding *49*, 447 (1990) (*corr. 52*, 513)
Wollastonite *42*, 145 (1987); *Suppl. 7*, 377 (1987)
Wood industries *25* (1981); *Suppl. 7*, 378 (1987)

X

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2,4-Xylidine	*16*, 367 (1978); *Suppl. 7*, 74 (1987)
2,5-Xylidine	*16*, 377 (1978); *Suppl. 7*, 74 (1987)
2,6-Xylidine (*see* 2,6-Dimethylaniline)	

Y

Yellow AB	*8*, 279 (1975); *Suppl. 7*, 74 (1987)
Yellow OB	*8*, 287 (1975); *Suppl. 7*, 74 (1987)

Z

Zearalenone (*see* Toxins derived from *Fusarium graminearum*, *F. culmorum* and *F. crookwellense*)	
Zectran	*12*, 237 (1976); *Suppl. 7*, 74 (1987)
Zinc beryllium silicate (*see* Beryllium and beryllium compounds)	
Zinc chromate (*see* Chromium and chromium compounds)	
Zinc chromate hydroxide (*see* Chromium and chromium compounds)	
Zinc potassium chromate (*see* Chromium and chromium compounds)	
Zinc yellow (*see* Chromium and chromium compounds)	
Zineb	*12*, 245 (1976); *Suppl. 7*, 74 (1987)
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