

BIOLOGICAL AGENTS

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A REVIEW OF HUMAN CARCINOGENS

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opinions of an IARC Working Group on the
Evaluation of Carcinogenic Risks to Humans,
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OF CARCINOGENIC RISKS
TO HUMANS

SCHISTOSOMA HAEMATOBIMUM

Schistosoma haematobium was considered by a previous IARC Working Group in 1994 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Taxonomy, structure, and biology

1.1.1 Taxonomy

Schistosomes are parasitic blood-dwelling fluke worms belonging to the genus *Schistosoma*; family, Schistosomatidae; order, Digenea; class, Trematoda; phylum, Platyhelminths; and kingdom, Animalia. The genus *Schistosoma* contains six species that are of major pathological importance to man, *Schistosoma haematobium* (*S. haematobium*), *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. guineensis* ([Webster et al., 2006](#)). The species differ in their final location in the human host, the species of the intermediate (snail) host they use in their life cycle, the pathology they induce, and the number, size and shape of the eggs they produce. This *Monograph* is restricted to *S. haematobium*.

1.1.2 Structure

Unlike all other pathologically important trematodes, schistosomes are not hermaphroditic, but have separate sexes. The adult worms are 1–2 cm long with a cylindrical body that features two terminal suckers, a complex tegument, a blind digestive tract, and reproductive

organs. The male's body forms a groove or gynaecophoric channel, in which it holds the longer and thinner female. As permanently embraced couples, the schistosomes live within the perivesical (*S. haematobium*) or mesenteric (other species) venous plexus. Schistosomes feed on blood particles through anaerobic glycolysis ([Rumnajek, 1987](#)).

1.1.3 Structure of the genome

The genome of *S. mansoni* is currently being sequenced and is almost completed; an Expressed Sequence Tag (EST) sequencing project has also started for *S. japonicum*, and *S. haematobium* ([El-Sayed et al., 2004](#)).

1.1.4 Life cycle and biology of the worm

The life cycle of *S. haematobium* is illustrated in Fig. 1.1. The female *S. haematobium* worm produces hundreds of eggs per day throughout her life. The eggs (144x58 µm, with a characteristic terminal spine) penetrate through the bladder wall where they are excreted with urine. Each ovum contains a ciliated larva (miracidium), which secretes proteolytic enzymes that help the eggs migrate into the lumen of the bladder. About half of the eggs produced do not reach the vesical lumen, and are carried

away with the bloodstream, and/or trapped in the tissues. These retained eggs provoke a granulomatous inflammatory response, which is the main cause of pathology in the human host. The excreted eggs hatch if they come into contact with water, and release the miracidium. These remain viable for up to 48 hours and are able to locate a suitable freshwater snail host (i.e. *Bulinus spp.* for *S. haematobium*) using external stimuli such as light and snail-derived chemicals. In the snail, asexual multiplication takes place, and several generations of multiplying larvae (sporocysts) develop. Eventually, these sporocysts produce large numbers of infective larvae with a typical bifurcated tail (cercariae). These leave the snail at a rate of thousands per day after a period of weeks. Shedding of these cercariae can continue for months. The cercariae survive for up to 72 hours and use water turbulence and skin-derived chemicals to locate the human host. They attach to and penetrate the human skin within 3–5 minutes. They lose their tail, and the young parasites (schistosomulae) migrate with the bloodstream via the lungs to the liver, where they mature into adult worms in the portal vein and mate. The paired worms migrate against the bloodstream to the perivesicular veins, where in a total of 4–7 weeks after infection they start producing eggs throughout their adult life. The lifespan of an adult worm averages 3–5 years, but can be as long as 30 years (Wilkins, 1987). An infected person probably harbours an average of hundreds (range, 10s–1000s) of worms (Gryseels & De Vlas, 1996).

1.2 Epidemiology of infection

1.2.1 Prevalence, geographic distribution

Human schistosomiasis is endemic in large areas of the (sub)tropics. It has been estimated that over 700 million people in 74 countries are exposed to the risk of schistosomal infection, and almost 200 million were estimated to be infected

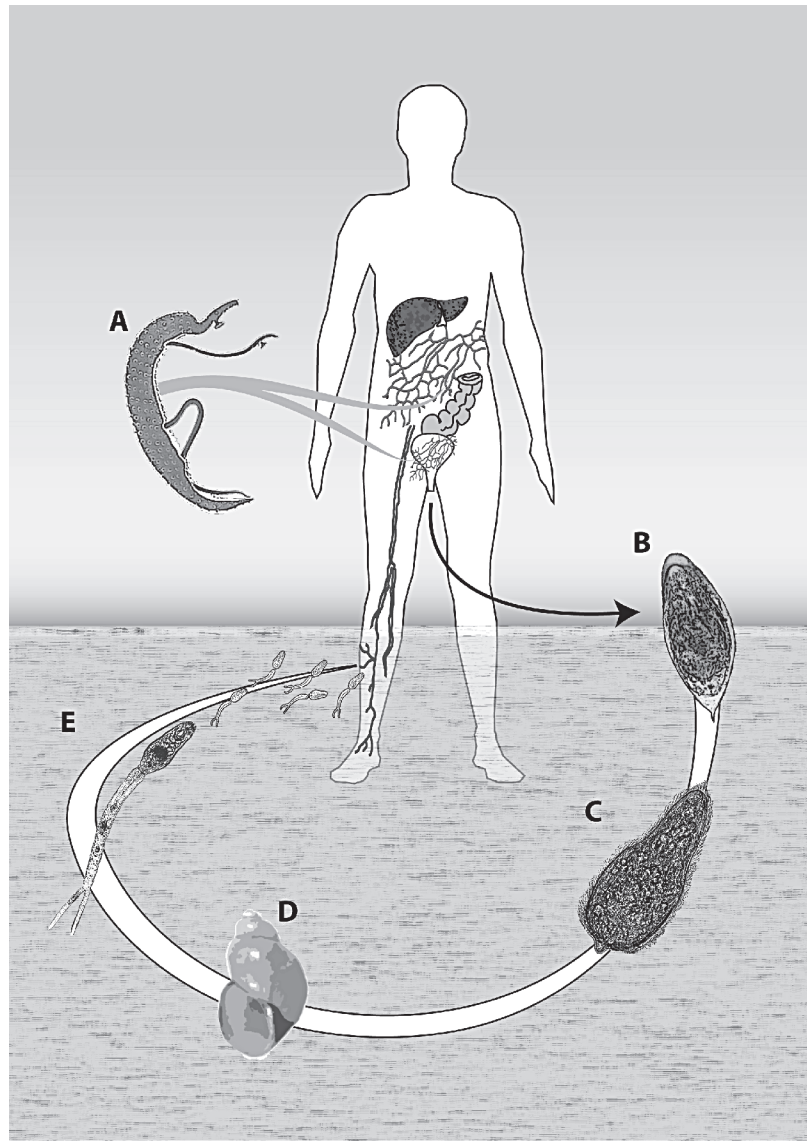
in 2003 (Fenwick, 2006), of which 85% in sub-Saharan Africa. About 95% of the cases are due to *S. mansoni* and *S. haematobium* infections. *S. haematobium* is endemic in 53 countries, in the Middle East and most of the African continent (Fig. 1.2; Chitsulo *et al.*, 2000).

Schistosomiasis is largely an infection found in rural areas, but urban schistosomiasis is an increasing problem in many countries (Mott *et al.*, 1990). Natural streams, ponds and lakes are typical sources of infection, but over the past few decades, man-made reservoirs and irrigation systems, as well as population growth and migration, have contributed to the spread of schistosomiasis (Gryseels *et al.*, 2006; McManus & Loukas, 2008).

Within countries, regions and villages, the distribution of schistosomiasis can be very focal, depending on variations in snail populations and human–water contact behaviour (Gryseels & Nkulikyinka, 1988). Also, the distribution of schistosomiasis can be highly uneven across individuals. The majority of the parasites are usually present in a small fraction of the infected individuals. Prevalence and intensities of infection generally show a typical convex-shaped curve with a peak at the ages of 5–15 years, and a decrease in adults. Sex-related patterns vary in relation to behavioural, professional, cultural, and religious factors (Jordan & Webbe, 1993).

1.2.2 Transmission, and risk factors for infection

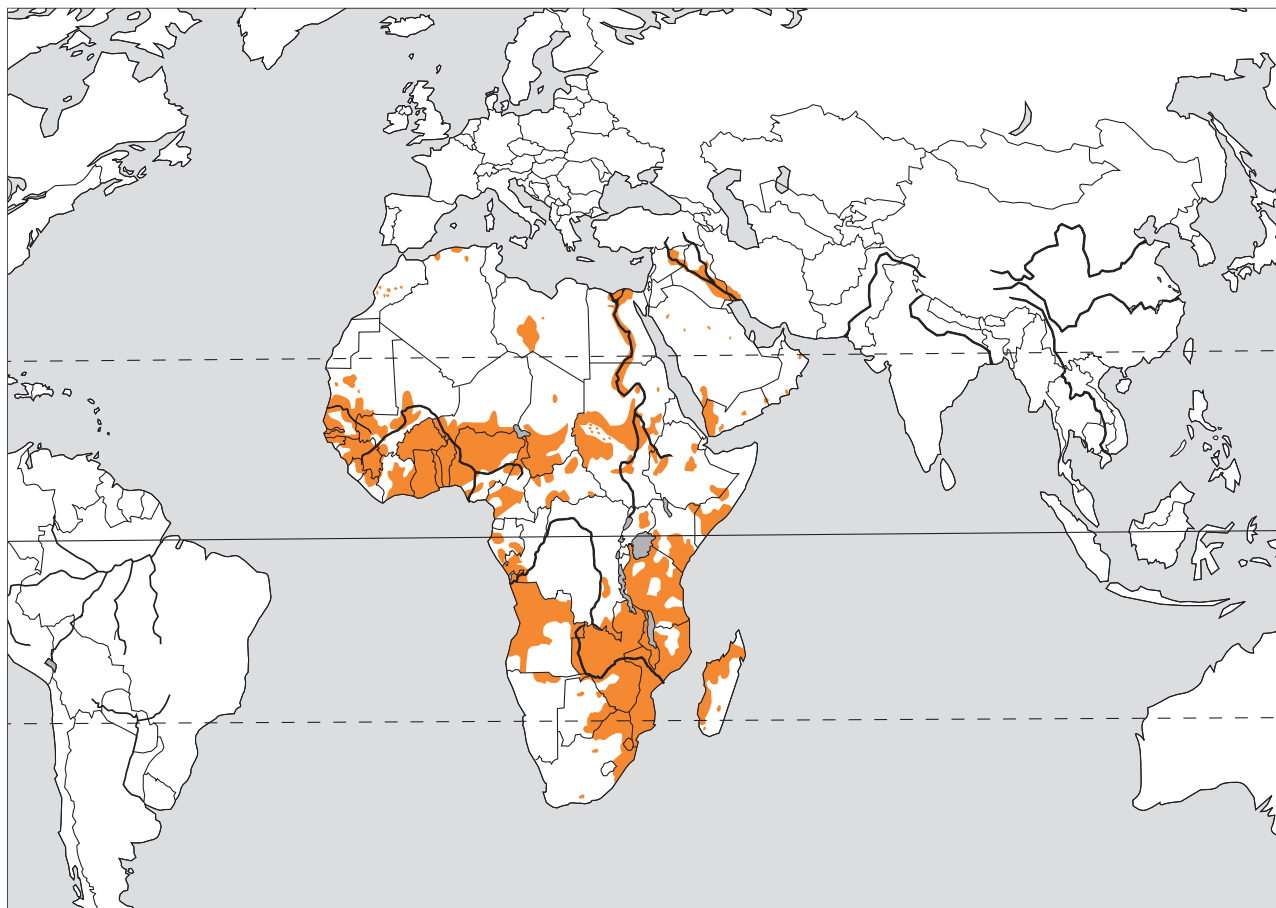
All *Schistosoma* infections follow direct contact with freshwater-harboured cercariae (see life cycle). Three major factors are responsible for maintaining the transmission of the infection: 1) contamination of fresh water with excreta containing schistosome eggs, 2) the presence of the snail intermediate hosts, and 3) human contact with water-infested with cercariae (Jordan & Webbe, 1993).

Figure 1.1 Life cycle of *Schistosoma haematobium*

A: paired adult worms; B: egg; C: miracidium; D: intermediate snail host; E: cercariae
Courtesy of Pr. Bruno Gryseels, Institute for Tropical Medicine, Antwerp, Belgium.

Contact with contaminated freshwater is the major risk factor of infection ([Jordan & Webbe, 1993](#)). The main risk groups are school-age children, specific occupational groups (fishermen, irrigation workers, farmers), and women and other groups using infested water for domestic purposes ([WHO Expert Committee, 2002](#)). Many other host-related and environmental risk factors have been identified that may affect the

risk of acquiring schistosome infection, and/or influence the distribution, prevalence, intensity of infection, morbidity and mortality of schistosomiasis. Among these are genetic factors ([Quinnell, 2003](#)), behaviour, household clustering ([Bethony *et al.*, 2001](#)), climate, immune response of the host, and concomitant infections (e.g. hepatitis) ([IARC, 1994](#)).

Figure 1.2 Global distribution of *Schistosoma haematobium*

Main foci *Schistosoma haematobium*: found mainly in sub-Saharan Africa, Nile valley in Egypt and Sudan, the Maghreb, and the Arabian peninsula.

Courtesy of Pr. Bruno Gryseels, Institute for Tropical Medicine, Antwerp, Belgium.

1.2.3 Persistence, latency, and natural history of infection

(a) Persistence

Schistosome worms do not multiply in the host. The infection status is the result of an accumulation of consecutive infections, where individuals with the most intense infections usually have a higher risk of developing morbidity ([Gryseels et al., 2006](#)). In the absence of re-infection, the infection subsides when the schistosome worm dies, which is usually after 3–5 years. However, in endemic areas with continuous exposure, re-infection is the rule rather than the exception.

In highly endemic areas, children start to accumulate worms as soon as they are old enough to have contact with water and may, because of the chronic nature of the infection and continued susceptibility to re-infection, remain infected throughout their lives.

The possibility that adults might develop immunity to schistosome infections was initially suggested based on the shape of the age–intensity curve in endemic communities, which characteristically shows a rise in intensity during the first two decades of life, followed by a decline in adults to very low levels ([Butterworth, 1998](#)). Indeed, numerous studies have provided epidemiological

and clinical evidence that people living in endemic areas acquire some form of protective immunity after years of exposure. However, age-related innate resistance mechanisms may also play an important part in the epidemiology of schistosomiasis ([Butterworth, 1993](#); [Gryseels et al., 2006](#)).

(b) Latency and natural history

Not much is known about the latency between the onset of infection and the appearance of cancer, nor about the steps that might lead to cancer.

Infection with *S. haematobium* is not synonymous with clinical disease, and many infections are asymptomatic. Of those infected, a small proportion develops serious chronic disease, after varying durations of exposure and infection ([Homeida et al., 1988](#); [Vennervald & Dunne, 2004](#)). [Mostafa et al. \(1999\)](#) noted that the incidence of bilharzial bladder cancer in various African countries peaks between the ages of 40–49 years, while infection with *S. haematobium* begins in childhood (as early as 6 months of age), and peaks usually in the second decade of life (between the ages of 5–15 years). This would imply a latency period of 20–30 years.

2. Cancer in Humans

2.1 Cancer of the urinary bladder

Earlier studies reported in the previous IARC Monograph ([IARC, 1994](#)) have supported an association between the occurrence of urinary bladder cancer and *S. haematobium* infection (for more recent reviews, see for example [Badawi et al., 1995](#); [Mostafa et al., 1999](#); [Mayer & Fried, 2007](#)). A substantial number of descriptive studies from Africa have shown that: the estimated incidence of urinary bladder cancer was higher in areas with a high prevalence of *S. haematobium* infection than in areas with a low prevalence; 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 more common in populations with a high prevalence of *S. haematobium* infection and a high proportion of urinary bladder cancers showing histological evidence of infection than in areas without these characteristics.

A large number of case series and case reports have repeatedly emphasized the prevalence of squamous cell urinary bladder tumours among patients with evidence of schistosomal infection. Clinically, the most notable and consistent feature described was the relatively young age of the cases that had evidence of a link to *S. haematobium* infection ([IARC, 1994](#)).

A more recent descriptive study by [Groeneveld et al. \(1996\)](#) on the incidence of different histological types of bladder cancer in various racial groups living within the same geographic area of Kwazulu-Natal, South Africa, reported similar results: squamous cell carcinoma occurred in 53% of the African patients (who have, according to the authors, a much higher risk of exposure and infestation to *S. haematobium* due to socio-economic, cultural and educational factors), and in 2% of the Caucasian patients. Moreover, eggs of *S. haematobium* were seen in microscopic sections of the bladder tumour in 85% of the patients with squamous cell carcinoma, and in 10% of the patients with transitional cell carcinoma. [The Working Group noted that no mention is made of the percentages of *S. haematobium* ova in microscopic sections of African patients with bladder cancer of the squamous-cell-carcinoma type].

The mean age at presentation of African patients was at least 20 years younger than that of Caucasian patients.

Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-09-Table2.1.pdf>) summarizes the case-control studies on the association between *S. haematobium* infection and urinary bladder cancer. A total of 7/8 case-control studies showed significant, positive associations between the occurrence of urinary bladder cancer and infection with *S. haematobium*, with estimated risks ranging from 2–15. The most recent study, from Egypt (Bedwani *et al.*, 1998), found that the odds ratio (OR) for urinary schistosomiasis was higher in subjects who were younger at first diagnosis (OR, 3.3 for <15 years), and with a long time since first diagnosis (OR, 3.0 for ≥35 years), suggesting a duration-risk relationship and a long-term effect of urinary schistosomiasis on bladder cancer (Bedwani *et al.*, 1998).

At the time of writing, no cohort studies on urinary bladder cancer and *S. haematobium* infections have been reported.

In contrast to some of the earlier case-control studies, all studies after 1994 considered possible confounding by age, sex, and smoking.

In one study that considered tobacco smoking as a confounding factor (Parkin *et al.*, 1994), no significant effect was observed due to tobacco smoking (OR, 1.1 for squamous cell bladder tumours).

In a study of schistosomiasis and the risk of bladder cancer in Egypt, Bedwani *et al.* (1998) assessed the interaction of history of urinary schistosomiasis with smoking. The interaction was significant ($P < 0.01$), with an odds ratio of 15.8 (95%CI: 5.1–48.4), and odds ratios for schistosomiasis only and for ever smoking only were 11.8 (95%CI: 2.8–50.1) and 13.8 (95%CI: 4.7–40.1), respectively.

2.2 Others

A number of studies have been conducted on the association of *S. haematobium* infection with other cancers, the results of which are summarized per cancer site below.

2.2.1 Cancers of the female genital tract

Other than urinary bladder cancer, cervical cancer and other malignancies of the female genitalia have been the most frequently reported cancers in association with *S. haematobium* infection, usually in the form of case reports (IARC, 1994). Recently, a number of additional cases of female genital malignancy in association with evidence of *S. haematobium* infection have been published (e.g. North *et al.*, 2003; Chenault & Hoang, 2006).

Two cross-sectional, one case-control and one pooled reanalysis studies have been published on the association between *S. haematobium* and cervical cancer (Wright *et al.*, 1982; Moubayed *et al.*, 1994; Parkin *et al.*, 1994; Riffenburgh *et al.*, 1997; Table 2.2, available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-09-Table2.2.pdf>). Parkin *et al.* (1994) consulted the same cancer registry from Zimbabwe as previously described for bladder cancer (Vizcaino *et al.*, 1994). Riffenburgh *et al.* (1997) pooled the data from the two earlier published studies. None of these studies showed a positive association between the risk for cervical cancer and infection with *S. haematobium*. [The Working Group noted that possible confounding by age, smoking, or human papillomavirus (HPV) was not considered in any of the studies.]

2.2.2 Other cancers

Eight cases of prostatic schistosomiasis associated with adenocarcinoma of the prostate have been reported, three of which were associated with *S. haematobium*, the others with *S. mansoni* or the parasite species was not specified (Cohen

[et al., 1995](#); [Bacelar et al., 2007](#)). [Cohen et al. \(1995\)](#) reported on three patients in South Africa (27–29 years of age) with advanced prostate cancer associated with the presence of multiple eggs of *S. haematobium* (some viable and others calcified).

Other malignancies that have been reported in association with *S. haematobium* infection include: squamous cell cancer of the female genitals, ovarian cystadenocarcinoma, teratoma and Brenner tumours, uterine leiomyosarcoma, male breast cancer, hepatocellular carcinoma, lymphoma, bladder sarcoma, rectal carcinoid tumour, and renal cell carcinoma. The number of patients with each malignancy is usually small, which precludes any evaluation of the data ([IARC, 1994](#); [Mayer & Fried, 2007](#)).

2.3 Impact of *Schistosoma* eradication

Although several authors have suggested prevention or control of *S. haematobium* as a way to manage schistosomiasis-associated bladder cancer ([Badawi et al., 1995](#); [Groeneveld et al., 1996](#); [Mostafa et al., 1999](#); [Mayer & Fried, 2007](#)), no such intervention study has been published so far. Some studies have investigated the effect of praziquantel, the drug of choice for the treatment of urinary schistosomiasis, on lesions in the urinary and genital tract ([Richter, 2003](#); [Kjetland et al., 2006](#)).

In a recent study of schistosomiasis-associated bladder cancer conducted in Egypt ([Koraitim et al., 1995](#)), both the proportion of transitional cell carcinoma (31% in 1960s versus 42% in late 1980s) and mean age at diagnosis (47 in 1960s versus 53 in late 1980s) in *Schistosoma*-positive cases had increased over time. Lower levels of schistosome infection (based on the number of eggs in the bladder wall) were observed among patients with bladder cancer in the late 1980s when compared to patients in the 1960s, which is likely to result from national efforts to control schistosomiasis and the availability of new drugs

with minimal side-effects ([Koraitim et al., 1995](#)). Transitional cell carcinomas were slightly more common among patients with low infection rates (56%), and squamous cell carcinomas were slightly more common (58%) among patients with moderate-to-high levels of infection ([Michaud, 2007](#)).

3. Cancer in Experimental Animals

Earlier studies reported in the previous *IARC Monograph* on infection with schistosomes ([IARC, 1994](#)) have examined experimental *S. haematobium* infections in mice, rats, hamsters, opossums, and non-human primates.

In mice, hamsters, and opossums, hyperplasia of the urinary bladder was observed. Urinary bladder tumours were also reported in one opossum ([IARC, 1994](#)). In several studies in non-human primates, hyperplasia and a few lesions described as tumours of the urinary bladder and ureter were observed. Overall, non-invasive papillary and nodular transitional cell carcinomas of the urinary bladder were observed in one talapoin monkey (*Cercopithecus talapoin*), five capuchin monkeys (*Cebus apella*), and one gibbon (*Hylobates lar*). These types of carcinomas were morphologically similar to tumours observed in human urinary bladder ([IARC, 1994](#); [Mostafa et al., 1999](#)).

In studies where animals infected with *S. haematobium* were treated with known urinary bladder carcinogens, an increase in urinary bladder tumour incidence was observed in infected mice administered 2-acetylaminofluorene, and in infected baboons (*Papio sp.*) treated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. The previous *IARC Monograph* Working Group commented on the short duration, the lack of verification of infection and inadequate documentation of experimental details for the study in mice, and that some of the diagnostic terms in

the report were difficult to interpret in the study in baboons ([IARC, 1994](#); [Mostafa et al., 1999](#)).

Since then, a new study ([Vuong et al., 1996](#)) has described histopathological findings following experimental *S. haematobium* infection in ten rodents including three marsh rats (*Holochilus brasiliensis*), and seven jirds (four *Meriones shawi* and three *M. Unguiculatus*). Comparisons were made with findings in two infected mice and six infected hamsters. All animals were about 3 months of age, and were exposed to 60–400 cercariae per animal by transcutaneous exposure through water (in an individual bath) containing the cercariae, except one marsh rat that received 750 cercariae transcutaneously on the abdominal skin. Eight rodents died naturally 117–256 days after infection (three marsh rats, three jirds, one mouse and one hamster); the remaining ten were sacrificed 100–195 days post infection. One marsh rat developed an in-situ squamous cell carcinoma of the urinary bladder (175 days post infection). Another marsh rat developed precancerous dysplastic changes of the bladder mucosa (123 days post infection). Only one jird (*Meriones shawi*) developed squamous metaplasia associated with mild-to-moderate dysplasia of the urothelial lining (117 days post infection). One mouse developed a squamous cell carcinoma of the urinary bladder (185 days post infection). No neoplastic or preneoplastic lesions were observed in the four hamsters.

4. Other Relevant Data

Several reviews have been published on the mechanisms of carcinogenesis induced by *S. haematobium* ([Rosin et al., 1994a, b](#); [Badawi, 1996](#); [Mostafa et al., 1999](#); [Herrera & Ostrosky-Wegman, 2001](#); [Mayer & Fried, 2007](#)). Several studies indicate that the carcinogenicity of *S. haematobium* is a multifactorial and multistage process where several

mechanisms are involved. *S. haematobium* eggs induce a chronic inflammation and irritation in the urinary bladder that seems to be associated with an increased initiation of cancer at the site of inflammation ([Rosin et al., 1994b](#)). The inflammatory response around the eggs gives rise to genotoxic factors and products that may cause genomic instabilities of host cells, leading to modifications in the regulation of tumour-suppressor genes and oncogenes as well as stimulating a proliferative response of the host cells to repair tissue damage caused by the inflammation ([Rosin et al., 1994a](#); [Badawi, 1996](#)). Furthermore, schistosomiasis-induced inflammatory cells have been shown to participate in the metabolic activation of procarcinogens, such as aflatoxins, and in the formation of carcinogenic nitrosamines from nitric oxide ([Rosin et al., 1994a](#); [Mostafa et al., 1999](#)).

The following is a review of the studies published since the previous *IARC Monograph* ([IARC, 1994](#)). The majority of the studies were conducted on bladder tissue samples from cancer patients with and without schistosomiasis.

4.1 Experimental data

4.1.1 Studies in vitro

(a) Mutagenicity

Neither worm nor egg extracts were shown to exhibit any *in vitro* mutagenicity by the *umu*-test or the V79/HGPRT assay with or without S9 mix ([Osada et al., 2005](#)).

(b) Effect on cell proliferation and on cell-cycle regulators

The effect of crude soluble egg antigen of *S. haematobium* on urothelial proliferation was tested on bovine endothelial cells (Endo), urothelial human transitional-cell carcinoma (J82), and human smooth muscle cell lines. Soluble egg antigen induced proliferation in a dose-dependent manner in both Endo and J82 cells

but not in smooth muscle cells. Furthermore, soluble egg antigen enhanced the expression of mRNA for the cell-cycle regulators, peripheral cell nuclear antigen and B-cell translocation protein (*BTG1*) in urothelial cells ([El-Awady et al., 2001](#)).

4.1.2 Studies in experimental animals

(a) Mitogenicity and carcinogenesis

The effect of *S. haematobium* infection and low doses of the bladder carcinogen *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine on the development of urothelial neoplasia was studied in a small group of baboons (*Papio sp.*). The study showed that proliferative and inflammatory changes occurred in infected animals not exposed to the carcinogen, whereas the addition of nitrosamine treatment induced neoplastic changes (carcinoma *in situ*). The carcinogen alone did not produce any changes ([Hicks, 1982](#)).

(b) Inhibition of glutathione-S-transferase (GST) activity in animals

Inhibition of GST activity may enhance the effect of many environmental carcinogens and toxic agents. The activity of GST, glutathione reductase (GR), and the levels of glutathione and free radicals were measured in different organs of male hamsters infected with *S. haematobium* 2, 4, 6, 8 and 10 weeks post infection. The total activity of GST was significantly increased in bladder tissue from infected animals when compared to uninfected controls 2, 4 and 6 weeks post infection ($P < 0.001$, $P < 0.025$ and $P < 0.001$, respectively). The GST activity was significantly decreased 8 and 10 weeks post infection ($P < 0.001$). The expression of GST isoenzymes was reduced in the kidney and bladder tissues at later stages (8 and 10 weeks) of the infection, and reduced in spleen and liver tissues at all infection stages. Free radicals (measured as thiobarbituric acid reactive substances) increased to high levels in the bladder, and to a lesser extent in other

organs ([Sheweita et al., 2003](#)). [The Working Group noted that the number of animals in the *S. haematobium* and the control groups was unspecified.]

4.2 Studies in exposed humans

The majority of the data concerning genotoxicity linked to *S. haematobium* infection are derived from studies on bladder tissue samples from cancer patients with schistosomiasis or a history of exposure to schistosomiasis. A representative sample covering various mechanisms such as DNA adducts, gene methylation, gene mutations, chromosomal alteration, expression of oncogenes and presence of oxidative stress markers are summarized below.

4.2.1 Production of *N*-nitroso compounds

Bacteria from a superimposed bacterial infection may reduce dietary nitrate to nitrite, which may react with compounds in urine to produce *N*-nitroso compounds. In a study in Egypt, higher ($P = 0.01$) levels of *N*-nitroso compounds and *N*-nitrosodimethylamine (NDMA) were found in the urine of individuals infected with *S. haematobium* compared to uninfected controls ([Abdel Mohsen et al., 1999](#)). [The Working Group noted that many of the study participants were smokers, that the history of contracting schistosomiasis infection was not available, and that the study did not include a control group with bacterial infection only.]

4.2.2 Oxidative stress markers

Levels of 8-hydroxy-2'-deoxyguanosine were markedly increased in bladder squamous cell carcinomas ($P = 0.001$) and transitional cell carcinomas ($P = 0.045$) associated with schistosomiasis when compared to non-schistosomiasis-associated cancers. This was accompanied by strong overexpression of DNA-repair genes

8-oxyguanine-DNA-glycosylase ($P=0.0047$) and apurinic/apyrimidinic endonuclease ($P=0.0121$); increased formation of DNA single strand breaks ($P=0.0106$); and higher inducible nitric oxide synthase ($P=0.022$), when comparing squamous cell carcinomas associated with schistosomiasis with non-schistosomiasis-associated cancers ([Salim et al., 2008](#)).

4.2.3 DNA adducts

DNA alkylation damage indicated by increased levels of O^6 -methyldeoxyguanosine has been demonstrated in tissue samples from schistosomiasis-associated bladder cancer patients. O^6 -alkylguanine-DNA alkyltransferase (ATase) activity was significantly higher in normal bladders than in bladders with cancers ($P<0.005$). A negative correlation between the levels of O^6 -methyldeoxyguanosine previously measured in the same samples and ATase activity was demonstrated ($r=-0.67$; $P<0.001$) ([Badawi et al., 1992, 1994](#)).

Paired samples from bladder tumour tissue and tissue without macroscopic signs of tumour invasion were collected in Egyptian patients. N^7 -methyldeoxyguanosine 3'-monophosphate (N^7 -MedGp) was measured by ^{32}P post-labelling. Levels of N^7 -MedGp were highly variable both in tumour and normal tissue and the mean difference in adduct levels between tumour and normal DNA was statistically significant. N^7 -MedGp levels were not associated with gender, age or presence of schistosomiasis ([Saad et al., 2006](#)). [The Working Group noted that the study had limited power with respect to examining the effect of schistosomiasis on the level of adducts because most of the patients were infected or had a history of infection.]

4.2.4 Gene methylation index

Methylation-specific polymerase chain reaction showed that schistosomiasis-associated bladder cancer samples had more genes methylated than non-schistosomal bladder cancer samples in a study in Egyptian patients ([Gutiérrez et al., 2004](#)).

4.2.5 Deletions and/or mutations in tumour-suppressor genes or oncogenes

Deletions and mutations in the $p16^{\text{INK4}}$ gene were found to be more frequent in schistosomiasis-associated bladder tumours from Egypt than in other bladder tumours from the Netherlands ([Tamimi et al., 1996](#)).

Expression of p53, Rb, EGFR and c-erbB-2 proteins was detected by immunohistochemistry and screened by single-strand conformation polymorphism and mutations in the *ras* (H, N, K) hotspots (12, 13, 61) and *p53* (exons 4–9) genes. Among 21 invasive bladder squamous cell carcinoma cases from South Africa infected with *S. haematobium*, positive staining for p53, EGFR and c-erbB-2 was reported in 38%, 67% and 28% of the tumours, respectively. Only one poorly differentiated tumour showed an absence of nuclear Rb staining. Changes in the *H-ras* gene were detected in three squamous cell carcinoma cases; two of which had mutations in *H-ras* codon 13 (gly→arg). The *H-ras* change most commonly seen in transitional cell carcinoma (gly→val change in codon 12) was not seen in any of the squamous cell carcinomas examined. The detection of multiple mutations at the *p53* locus in the schistosomiasis-related cancers suggests the involvement of a specific carcinogenic agent, possibly nitrosamines ([Ramchurren et al., 1995](#)).

Mutations in the *p53* gene were assessed in tissue specimens from 92 Egyptian bladder cancer patients from an area hyperendemic for schistosomiasis. About 90% of the patients had a history of schistosomiasis and/or evidence of

schistosome eggs adjacent to the carcinoma. Thirty patients had mutations in exons 5–8 of the *p53* gene. Of 19 mutations in squamous cell carcinoma, 16 were base-pair substitutions, two were deletions, and one an insertion. All the mutations in transitional cell carcinoma were base-pair substitutions. Combining the 33 mutations from this study with six obtained from another study ([Habuchi et al., 1993](#)) of Egyptian schistosomiasis-associated squamous cell carcinoma, a mutational spectrum was compiled and compared with a non-schistosomal bladder cancer spectrum assembled from 118 mutations reported in the literature. The proportion of base-pair substitutions at CpG dinucleotides was significantly higher (18/34 versus 25/103, $P=0.003$) in schistosomiasis-associated bladder cancer than in non-schistosomal bladder cancer ([Warren et al., 1995](#)).

[The Working Group reported that several studies compared tumours from schistosomiasis-infected patients to those from countries not endemic for schistosomiasis (e.g. [Shaw et al., 1999](#); [Swellam et al., 2004a](#)). These studies were not reviewed because of the difficulty of ascribing differences to schistosomiasis or to other factors that vary between these countries.]

4.2.6 Expression of oncogenes

A significant correlation was recognized between bcl-2 overexpression and bladder squamous cell carcinoma with schistosomiasis. Bcl-2 expression was 74.8 U/mg protein in squamous cell carcinoma and 45.2 U/mg protein in transitional cell carcinoma. It was 82.41 U/mg protein in schistosomiasis cases compared to 35.8 U/mg protein in non-schistosomiasis cases ([Swellam et al., 2004b](#)).

4.2.7 Biomarkers of bladder cancer

The detection of the biomarkers associated with bladder cancer BLCA-4 (a nuclear matrix protein involved in gene regulation and produced only in neoplastic bladder cells) and quantitative nuclear grading was performed in a population-based study from an area in Ghana endemic for *S. haematobium*. The results showed a close correlation between BLCA-4, quantitative nuclear grading and severe bladder damage such as bladder wall masses and polyps. The overall prevalence of BLCA-4 positivity was 40%. A total of 62/73 cytopathology Papanicolaou-stained smears were seen to have squamous metaplasia ([Shiff et al., 2006](#)).

4.2.8 Effect of *S. haematobium* infection on detoxifying enzymes

S. haematobium infection has been found to markedly decrease the activity of the carcinogen-metabolizing enzymes GST and NDMA-N-demethylase in human bladder cancer tissue ([Sheweita et al., 2004](#)). [The Working Group noted that this may change the capacity of the bladder to detoxify many endogenous compounds, and may potentiate the effects of bladder carcinogens such as *N*-nitrosamines.]

4.3 Host susceptibility

Cytological abnormalities in urine sediment were investigated in a cross-sectional survey of a population (1014 individuals aged 1–91 years) living in an area in Kenya endemic for *S. haematobium*. No cancers were detected in the study population. The prevalence of inflammation (39%), hyperkeratosis (30%), metaplasia (33%) and frank atypia (0.4%) was much higher than what had been reported from non-endemic areas. *S. haematobium* infection was strongly associated with an increased risk of metaplasia or hyperkeratosis (relative risk > 2.8-fold, $P < 0.001$).

Among children, the incidence of metaplasia was linked with concurrent schistosome infection, whereas older individuals tended to have metaplasia incidences independent of the level of infection or inflammation. The incidence of moderate or severe metaplasia displayed two age-related peaks; one among the age group 10–14 years, where the intensity of infection is highest, and a second among individuals over 60 years of age ([Hodder et al., 2000](#)).

4.4 Synthesis

It is well established that *S. haematobium* with egg deposition in the tissue leads to severe inflammation of the urinary bladder wall with accumulation of inflammatory cells resulting in increased oxidative stress. Overall, the studies summarized above suggest that the observed increased levels of oxidative stress in the schistosomiasis-associated bladder carcinomas correlate with genotoxicity and activation of repair genes, and point towards a relationship between oxidative stress induced by continuous and chronic inflammation due to schistosome infection and possibly nitric-oxide-mediated DNA genotoxicity. The excess of DNA alterations could result from nitric oxide produced by the inflammatory response provoked by *Schistosoma* eggs, and alkylation of DNA by *N*-nitroso compounds.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Schistosoma haematobium*. Chronic infection with *Schistosoma haematobium* causes cancer of the urinary bladder.

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

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

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