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.]