HYDROXYUREA

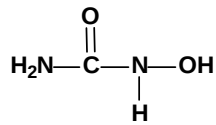
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 127-07-1
Chem. Abstr. Name: Hydroxyurea
IUPAC Systematic Name: Hydroxyurea
Synonyms: *N*-(Aminocarbonyl)hydroxylamine; carbamohydroxamic acid; carbamohydroximic acid; carbamoyl oxime; HU; hydroxycarbamide; hydroxycarbamine; hydroxylurea

1.1.2 *Structural and molecular formulae and relative molecular mass*



$CH_4N_2O_2$ Relative molecular mass: 76.06

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White, odourless, crystalline (needles) powder (Gennaro, 1995; Budavari, 1996)
(*b*) *Melting-point*: 133–136 °C (Budavari, 1996)
(*c*) *Spectroscopy data*: Infrared (prism, [45287; 475C]; grating [30287]; FT-IR, [801C]) and nuclear magnetic resonance (proton, [33625; 671D]; C-13, [24474]) spectral data have been reported (Pouchert, 1981, 1983, 1985; British Pharmacopoeial Commission, 1993; Sadtler Research Laboratories, 1995).
(*d*) *Solubility*: Very soluble in water; slightly soluble in ethanol (Budavari, 1996; American Hospital Formulary Service, 1997)
(*e*) *Stability*: Hygroscopic and decomposes in the presence of moisture (Royal Pharmaceutical Society of Great Britain, 1999)

1.1.4 *Technical products and impurities*

Hydroxyurea is available as a 200-, 300-, 400- or 500-mg capsule; the capsule may also contain calcium citrate, citric acid, colourants (D&C Red No. 28; D&C Red No. 33; D&C Yellow No. 10; FD&C Blue No.1; FD&C Green No. 3; FD&C Red No. 40), disodium citrate, erythrosine, gelatin, indigocarmine, iron oxide, lactose, magnesium stearate, sodium lauryl sulfate, sodium monohydrogen phosphate, tartrazine and titanium dioxide (American Hospital Formulary Service, 1997; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998; Editions du Vidal, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998; US Pharmacopeial Convention, 1998; Medical Economics Data Production, 1999).

Trade names for hydroxyurea include Biosupressin, Droxia, Droxiurea, Hidroks, Hidroxiurea Asofarma, Hidroxiurea Filaxis, Hidroxiurea Martian, Hydrea, Hydrea capsules, Hydreia, Hydroxycarbamid, Hydroxycarbamide capsules BP 1998, Hydroxycarbamide capsules USP 23, Hydroxyurea, Litalir, Onco-Carbide, Oxyurea and Syrea (CIS Information Services, 1998; Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

1.1.5 *Analysis*

Several international pharmacopoeias specify infrared absorption spectrophotometry with comparison to standards as the method for identifying hydroxyurea; titration with sodium thiosulfate is used to assay its purity. Similar methods are used for identifying and assaying hydroxyurea in pharmaceutical preparations (British Pharmacopoeial Commission, 1993; US Pharmacopeial Convention, 1994).

1.2 Production

Hydroxyurea has been prepared by the reaction of calcium cyanate with hydroxylamine nitrate in absolute ethanol and by the reaction of potassium cyanate and hydroxylamine hydrochloride in aqueous solution. Hydroxyurea has also been prepared by converting a quaternary ammonium anion exchange resin from the chloride form to the cyanate form with sodium cyanate and reacting the resin in the cyanate form with hydroxylamine hydrochloride (Graham, 1955).

Information available in 1999 indicated that hydroxyurea was manufactured and/or formulated in 25 countries (CIS Information Services, 1998; Royal Pharmaceutical Society of Great Britain; 1999; Swiss Pharmaceutical Society, 1999).

1.3 Use

Hydroxyurea is a chemically simple antimetabolite, which is cytostatic by inhibiting ribonucleotide reductase, an enzyme important in creating deoxynucleosides for DNA

replication in growing cells (Gao *et al.*, 1998). Hydroxyurea was initially synthesized over 120 years ago, but its potential biological significance was not recognized until 1928. In the late 1950s, the drug was evaluated in a large number of experimental murine tumour systems and shown to be active against a broad spectrum of tumours. Phase I trials with hydroxyurea began in 1960 and by the late 1960s it was in clinical use (Donehower, 1992).

Hydroxyurea has been used, or investigated for use, in the treatment of a number of diseases.

(*a*) *Sickle-cell haemoglobinopathy*

Hydroxyurea is widely used to treat severe sickle-cell disease (Charache *et al.*, 1996; Ferster *et al.*, 1996; de Montalembert *et al.*, 1997) and beta-thalessaemia–sickle-cell disease (Voskaridou *et al.*, 1995). It was shown to induce fetal haemoglobin synthesis (Fibach *et al.*, 1993; Maier-Redelsperger *et al.*, 1998), and preliminary reports demonstrated its benefit in beta-thalessaemia–sickle-cell haemoglobinopathy (Loukopoulos *et al.*, 1998). The efficacy of hydroxyurea in sickle-cell disease is well validated, but its use appears to be limited to patients with frequent crises and hospitalizations. The doses used in patients with sickle-cell anaemia are 25 mg/kg bw per day in children (Ferster *et al.*, 1996) and up to 35 mg/kg bw per day in adults (Charache *et al.*, 1996).

(*b*) *Myeloproliferative syndromes*

Hydroxyurea is also used as a cytostatic agent in myelodyplastic or myeloproliferative diseases, including chronic myeloid leukaemia (Donehower, 1992; Fitzgerald & McCann, 1993; Guilhot *et al.*, 1993), polycythemia vera (Najean & Rain, 1997a,b), myelodysplastic syndrome (Nair *et al.*, 1993) and essential thrombocythaemia and corticosteroid-resistant hypereosinophilia (Donehower, 1992). The risks and benefits of hydroxyurea in haematological disease are debated and have been reviewed (Donehower, 1992). It is used most commonly in chronic myeloid leukaemia to prevent or delay the onset of blast crises, and complete responses have been seen occasionally (Tanaka *et al.*, 1997).

(*c*) *With didanosine in the treatment of HIV/AIDS*

Hydroxyurea is given as an adjunct with didanosine (see monograph, this volume) in treatment of human immunodeficiency virus (HIV) infection. Several case series and randomized trials have shown dramatic results with the combination (Biron *et al.*, 1996; Montaner *et al.*, 1997; Foli *et al.*, 1998), although not without exception (Simonelli *et al.*, 1997). Trials in which didanosine and hydroxyurea were given in combination with other agents (Lisziewicz *et al.*, 1998; Rutschmann *et al.*, 1998) led to large-scale controlled trials which are currently under way. Intriguing reports of prolonged periods without rebound viraemia after didanosine and hydroxyurea therapy, especially in

patients who began treatment shortly after HIV infection, have led to intensive investigation (Vila *et al.*, 1997; Lisziewicz *et al.*, 1998). The doses of hydroxyurea used in combination with didanosine are 500–1000 mg/day (Montaner *et al.*, 1997; Rutschmann *et al.*, 1998).

Hydroxyurea itself has no antiviral effect (Lori *et al.*, 1997a). Its mechanism of action with didanosine appears to be selective inhibition of ribonucleotide reductase, thus decreasing endogenous dATP concentrations, leading to increased generation of dATP from the pro-drug didanosine. Hydroxyurea thus improves the antiviral potency of didanosine (Lori *et al.*, 1997b; De Boer *et al.*, 1998; Johns & Gao, 1998). The effect is likely to be greater with dATP analogues such as didanosine than with other antiviral nucleoside analogues (Gandhi *et al.*, 1998).

Increased cytokine levels (Navarra *et al.*, 1995, 1996) and adrenal activity have been seen in hydroxyurea-treated patients (Navarra *et al.*, 1990, 1998) but not in HIV-infected patients treated with hydroxyurea and didanosine.

(*d*) *Psoriasis*

Hydroxyurea can be administered over long periods to treat psoriasis (Moschella & Greenwald, 1973; Boyd & Neldner, 1991), although it is currently used relatively infrequently.

(*e*) *Solid tumours*

Hydroxyurea is used as a radiosensitizing agent in carcinoma of the cervix (Stehman, 1992; Stehman *et al.*, 1997) and glioma (Levin & Prados, 1992). Other malignancies in which use of hydroxyurea as an adjunct has been studied (Wadler *et al.*, 1996) include meningioma (Schrell *et al.*, 1997), uterine leiomyosarcoma (Currie *et al.*, 1996a) and uterine mixed mesodermal tumours (Currie *et al.*, 1996b).

1.4 Occurrence

Hydroxyurea is not known to occur as a natural product. No data on occupational exposure were available to the Working Group.

1.5 Regulations and guidelines

Hydroxyurea is listed in the British, French and US pharmacopoeias (Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

2. Studies of Cancer in Humans

The carcinogenic potential of hydroxyurea has been studied in patients with chronic myeloproliferative disorders, which include chronic myeloid leukaemia, ideopathic myelofibrosis, polycythaemia vera and essential thrombocythaemia. An assessment of the carcinogenicity of this agent is hampered by an inherent tendency of chronic myeloproliferative disorders to undergo spontaneous transformation to myelodysplastic syndrome or acute leukaemia. Thus, among 431 patients with polycythaemia vera who were randomized to one of three treatment arms, phlebotomy (n = 134), chlorambucil (n = 141) or radioactive phosphorus (n = 156), patients in the phlebotomy arm were found to have a cumulative risk for acute leukaemia after 11–18 years of follow-up of 1.5% on the basis of two observed cases. The rates for acute myeloid leukaemia in the US population indicate that about 0.13 cases would have been expected (Landaw, 1986).

Although hydroxyurea is used in the treatment of sickle-cell anaemia and of psoriasis, these conditions are not suspected to predispose to cancer.

2.1 Case reports

Several case reports have been published on the occurrence of multiple squamous-cell and basal-cell carcinomas of the skin in patients who received prolonged treatment with hydroxyurea for chronic myeloid leukaemia (Disdier *et al.*, 1991; Stasi *et al.*, 1992; Best & Petitt, 1998; De Simone *et al.*, 1998), essential thrombocythaemia or polycythaemia vera (Callot-Mellot *et al.*, 1996; Best & Petitt, 1998). The skin carcinomas were typically seen in sun-exposed areas and had been preceded by other degenerative cutaneous manifestations.

Reiffers *et al.* (1985), van den Anker-Lugtenburg and Sizoo (1990) and Furgerson *et al.* (1996) described the occurrence of acute leukaemia in patients who had been treated with hydroxyurea for long periods for essential thrombocythaemia and had not received other treatments.

2.2 Cohort studies

These studies are summarized in Table 1.

2.2.1 *Polycythaemia vera* (see also section 2.2.3)

Sharon *et al.* (1986) reported the results of a prospective study conducted in Israel of 36 patients with polycythaemia vera who were treated with hydroxyurea at a daily dose of 500–1500 mg for 1–5.6 years. Nineteen of the patients had previously been treated with other myelosuppressive drugs. During treatment, no cases of leukaemia or other malignant neoplasms were seen.

Table 1. Cohort studies of acute leukaemia (AL) and myelodysplastic syndrome (MDS) in patients treated with hydroxyurea

Country (reference)	Study design (no. of cases); period	Daily dose (mg/kg bw)[a]	Follow-up (years) average (range)	Cases of AL and MDS	Proportion with blast transformation	Comments
Polycythaemia vera						
Israel (Sharon *et al.*, 1986)	Prospective (36); not reported	[7–21]	Duration of treatment (1–5.6)	AL: 0	0%	19 patients with other prior myelosuppressive treatment
USA (West, 1987)	Prospective (100); 1963–83	[10]	Duration of treatment 5.4 (0.3–18)	AL: 1	1%	Possibly one additional case of chronic leukaemia
USA (Kaplan *et al.*, 1986)	Prospective (51); 1979–86 (Nand *et al.*, 1990)	~ 15	Duration of treatment 4.7 (0.1–7.5)	AL: 3	5.9%	Another case of AL diagnosed 19 years after first diagnosis (Holcombe *et al.*, 1991)
USA (Nand *et al.*, 1990)	Retrospective (18); 1975–87	1.7 ± 1.7 g/day, 1 year	Duration of treatment: 12	AL: 5	27.8%	10 patients also received other myelosuppressive treatment.
Sweden (Weinfeld *et al.*, 1994)	Prospective (21); since 1976	[7–21]	(5–> 10)	AL: 3	14%	Patients considered in a study of MDS
USA (Nand *et al.*, 1996)	Cross-sectional (16); 1993–95; prevalent cases	1.5 (0.5–6) g/day, 1 year	5.5 (1.5–20)	AL or MDS: 1	6.2%	Patients considered in a study of MDS
France (Najean & Rain, 1997a)	Prospective (150), since 1980	10–15	Follow-up (1–17)	AL: not given MDS: not given	Actuarial risk: 10% at 13th year	Cases of AL and MDS not distinguished according to treatment received

Table 1 (contd)

Country (reference)	Study design (no. of cases); period	Daily dose (mg/kg bw)[a]	Follow-up (years) average (range)	Cases of AL and MDS	Proportion with blast transformation	Comments
Essential thrombocythaemia						
France (Belluci *et al.*, 1986)	Retrospective (42); 1961–82	Not reported	Follow-up (> 0–19)	AL: 2	4.8%	
France (Liozon *et al.*, 1997)	Retrospective (53); 1981–95	4–43	Follow-up (6)	AL: 1 MDS: 2	5.7%	
France (Sterkers *et al*., 1998)	Retrospective (251); 1970-91	[21] (starting dose)	Follow-up; 8.2 (1.8–22)	AL: 5 MDS: 9	5.6%	50 patients also received other myelosuppressive treatment
Sweden (Löfvenberg *et al.*, 1990)	Prospective (32); 1981–89	15–20	Treatment duration; 3.8 (1.3–6.6)	AL: 2 MDS: 1	9.4%	Patients considered in a study of MDS
Sweden (Weinfeld *et al.*, 1994)	Prospective (9); since 1976	[7–21]	(5–> 10)	AL: 1	11%	Patients considered in a study of MDS
Chronic myeloproliferative disease						
Sweden (Löfvenberg *et al.*, 1990)	Prospective (81) 1981–89	15–20	Treatment duration; 3.9 (0.1–8.7) (means)	AL: 3 MDS: 1	4.9%	3 patients with other prior myelosuppressive treatment, but none of AL or MDS patients
Sweden (Weinfeld *et al.*, 1994)	Prospective (50); since 1976	7–21	(5–> 10)	AL: 9 MDS: 1	20%	13 patients with other prior myelosuppressive treatment

Table 1 (contd)

Country (reference)	Study design (no. of cases); period	Daily dose (mg/kg bw)[a]	Follow-up (years) average (range)	Cases of AL and MDS	Proportion with blast transformation	Comments
Chronic myeloproliferative disease (contd)						
USA (Nand *et al.*, 1996)	Cross-sectional (25); prevalent cases; 1993–95	0.25–6 g/day, 1 year	5.2 (0.3–20)	AL or MDS: 2	8.0%	
Sickle-cell anaemia						
USA (Charache *et al.*, 1995)	Prospective (152); 1992–94	0–35	1.8 (1.2–2.0)	AL: 0	0%	Treatment arm in a clinical trial
USA (Scott *et al.*, 1996)	Prospective (13); 1992–95	10–35	Treatment: 2.0 (0.5–3.3)	AL: 0	0%	
Cyanotic congenital heart disease						
France (Triadou *et al.*, 1994)	Prospective (64); not reported	10	5 (2–15)	AL: 0	0%	

[a] When not given in the paper, calculated assuming a body weight of 70 kg

West (1987) studied the incidence of acute leukaemia in 100 patients in Kentucky, USA, who were treated for polycythaemia vera which had been diagnosed during 1963–83. They were also treated by phlebotomy. The mean daily dose of hydroxyurea was 720 mg, and the duration of therapy ranged from three months to 18 years (mean, 5.4 years). During this time, two (2%) cases of leukaemia were observed: one chronic neutrophilic leukaemia after nine months of treatment and one acute myeloid leukaemia after five years. The authors noted that the chronic neutrophilic leukaemia might have been present before the date of recruitment.

Of 118 patients in New York, USA, with polycythaemia vera, all had received supplementary phlebotomy and hydroxyurea at a daily dose of 30 mg/kg bw for one week, then 15 mg/kg bw and then modified downwards and upwards; 59 of the patients had had no prior myelosuppressive therapy (Donovan *et al*., 1984). Hydroxyurea was given for a mean of 4.7 years (range, one month to 7.5 years). Three out of 51 patients with no prior myelosuppressive therapy developed acute leukaemia after 1.7, 2.8 and 4.8 years of treatment (Kaplan *et al*., 1986). Two cases of leukaemia (1.5%) were observed in the most appropriate historical control group of 134 patients who had been treated exclusively with phlebotomy (Landaw, 1986), and the difference between the two groups was not statistically significant. [The Working Group noted the reduction in numbers of hydroxyurea-treated patients from 59 to 51, which was unexplained.] In a subsequent case report, Holcombe *et al*. (1991) described an additional case of chronic myelomonocytic leukaemia (which transforms to acute myeloid leukaemia) in the group of 51 hydroxyurea-treated patients with polycythaemia vera. This case was seen 19 years from the date of initial diagnosis. No data were given on the historical control group.

Nand *et al*. (1990) conducted a retrospective study of 48 patients in Chicago, Illinois, USA, with polycythaemia vera diagnosed during 1975–87 (seven cases were diagnosed previously) and treated over a period of 12 years. Of these, 18 had been treated with hydroxyurea at doses ranging from 500 mg every other day for nine months to 1000 mg daily for seven years. Four cases of acute leukaemia were seen among 10 of the patients who had received hydroxyurea in combination with other myelosuppressive treatment including radioactive phosphorus and one case among eight patients who had received hydroxyurea alone (relative risk = 3.8; $p = 0.38$). The cases developed at a mean of 3.9 years after the start of treatment. [The Working Group noted the small size of the study.]

Two published reports are available on the results of a clinical trial of the French Polycythaemia Vera Study Group (Najean *et al*., 1996; Najean & Rain, 1997a). The most recent report includes 292 patients with polycythaemia vera diagnosed after 1980 when the patients were aged 0–64 years. The patients were treated with either hydroxyurea ($n = 150$) at a daily dose of 25 mg/kg bw followed by a maintenance dose of 10–15 mg/kg bw, or pipobroman [1,4-bis(3-bromopropionyl)piperazine] ($n = 142$). The patients were followed for 1–17 years, during which time nine cases of acute myeloid leukaemia and four cases of myelodysplastic syndrome were observed. The

precise treatment received in these 13 cases was not specified, but the authors stated that the actuarial risk for acute myeloid leukaemia or myelodysplastic syndrome was about 10% at the 13th year of follow-up, with no difference according to treatment. Four cases of non-melanoma skin cancer were seen in patients given hydroxyurea only and one in a patient given pipobroman only, while six cancers at sites other than the bone marrow and non-melanoma skin were seen in six patients given hydroxyurea and five given pipobroman. These frequencies of extracutaneous solid tumours were only slightly greater than those expected for this age group.

In a complementary trial covering the period 1979–96, Najean and Rain (1997b) recruited 461 patients with polycythaemia vera who were over 65 years of age and had not previously been treated with chemotherapeutic agents. Initially, the patients were treated with radioactive phosphorus (0.1 mCi/kg bw with a maximum of 7 mCi) administered intravenously until complete remission of the polycythaemia was obtained. They were then randomized to receive either maintenance treatment with low-dose hydroxyurea (5–10 mg/kg bw per day) (n = 219) or simple surveillance (n = 242). When the haematocrit of a patient in either treatment arm had increased to 50% and the erythrocyte volume was > 125% of the normal value during follow-up, intravenous administration of radioactive phosphorus was resumed. The median survival was 9.1 years in the group receiving maintenance therapy and 11.2 years in the surveyed group (p = 0.10). In a subset of 408 patients followed for more than two years (maximum follow-up time, 16 years), the mean annual dose of radioactive phosphorus was 0.009 mCi/kg bw in the group receiving hydroxyurea and 0.033 mCi/kg bw in the surveyed group [average for all study subjects, approximately 0.021 mCi/kg bw per year]. In the same subset of 408 patients, 41 haematological malignancies were observed, consisting of 15 cases of acute myeloid leukaemia, two of non-Hodgkin lymphoma, two of chronic lymphocytic leukaemia, two of multiple myeloma, three of chronic myelomonocytic leukaemia and 17 of myelodysplastic syndrome. The precise treatment schedule received in these 41 cases was not specified, but the authors stated that statistical analysis (log-rank test) showed a significantly increased risk for these tumour types combined in patients receiving maintenance treatment with hydroxyurea when compared with those under simple surveillance (p = 0.01 or p = 0.03, depending on whether outcomes were analysed according to intention to treat or the main therapy received). The dose of radioactive phosphorus received by the patients who developed leukaemia was moderately higher (0.044 mCi/kg bw per year) than that received by other patients (0.032 mCi/kg bw per year), but the difference was not statistically significant. Seven cases of non-melanoma skin cancer were observed among patients receiving hydroxyurea maintenance and two cases in the surveyed group. [The Working Group noted that the average dose of 0.032 mCi/kg bw per year received by persons without leukaemia does not concord with the average level of 0.021 mCi/kg bw per year for all study subjects followed for more than two years. The Group also noted that the distributions of haematological malignancies by type of treatment were not presented and that the cases of non-Hodgkin lymphoma, chronic lymphocytic leukaemia and

multiple myeloma in these elderly people were apparently grouped with the cases of acute myeloid leukaemia and myelodysplastic syndrome before risk analyses were performed, which limits interpretation of the results.]

2.2.2 *Essential thrombocythaemia* (see also section 2.2.3)

Bellucci *et al.* (1986) reviewed the medical records of 94 patients (average age, 49.5 years; range, 6–90) in one treatment centre in Paris, France, in whom essential thrombocythaemia had been diagnosed during 1961–82. The patients were followed up for periods ranging from a few months to 19 years, during which time five cases of acute leukaemia were observed. Two of the five cases occurred in the subgroup of 42 patients who had received hydroxyurea as the only chemotherapeutic agent.

In a treatment centre in Limoges, France, Liozon *et al.* (1997) conducted a retrospective follow-up study of 58 patients (mean age, 66.5 years; range, 18–85) in whom essential thrombocythaemia had been diagnosed during 1981–95. The mean duration of follow-up was approximately five years. Among the 53 patients who had received hydroxyurea as first-line therapy (mean weekly dose, 6 g; range, 2–21 g), one developed acute myeloid leukaemia, one developed chronic myelomonocytic leukaemia and one had myelodysplastic syndrome.

Sterkers *et al.* (1998) reviewed the medical records of 357 patients (median age, 62 years; range, 30–75) with essential thrombocythaemia diagnosed between 1970 and 1991 and followed-up until 1996 at two haematological centres, in Lille and Lomme, France. Overall, 326 of the 357 patients had been treated with at least one chemotherapeutic agent, and 251 had received hydroxyurea at a starting dose of 1.5 g/day (some had received pipobroman). Within a median duration of follow-up of 8.2 years (range, 1.8–22 years), six patients had developed acute myeloid leukaemia and 11 myelodysplastic syndrome (including chronic myelomonocytic leukaemia). Fourteen of these 17 patients had received hydroxyurea at some time, while seven of the cases were seen in the subgroup of 201 patients who had been treated with hydroxyurea alone.

2.2.3 *Chronic myeloproliferative disease*

In one treatment centre in Sweden, 81 consecutive patients (age range, 31–82 years) with Philadelphia chromosome-negative chronic myeloproliferative disease, consisting of 35 cases of polycythaemia vera, 32 of essential thrombocythaemia, 12 of myelofibrosis and two of myeloproliferative syndrome [not further specified], were followed prospectively from 1981 to 1989 (Löfvenberg & Wahlin, 1988; Löfvenberg *et al.*, 1990). All had received maintenance treatment with hydroxyurea at a dose of 15–20 mg/kg bw per day. During an average follow-up of 3.9 years (range, one month to 8.7 years), three cases of acute myeloid leukaemia (two cases in patients with essential thrombocythaemia, one in a patient with myelodysplastic syndrome) and one

case of myelodysplastic syndrome (in a patient with essential thrombocythaemia) were seen. None of these four cases had been treated with alkylating agents or radioactive phosphorus before treatment with hydroxyurea. No data were available on the number of cancers to be expected among these patients on the basis of incidence rates in the general population.

In another treatment centre in Sweden, Weinfeld *et al.* (1994) conducted a prospective follow-up of 50 consecutive patients (age range, 33–82 years) with Philadelphia chromosome-negative chronic myeloproliferative disease, consisting of 30 patients with polycythaemia vera, 10 with essential thrombocythaemia and 10 with myelofibrosis, of whom 21, 9 and 7, respectively, had been treated only with hydroxyurea at 60 mg/kg per day for the first week and then 0.5–1.5 g/day. The median observation period was > 10 years, and the minimum was five years, during which time nine cases of acute leukaemia and one case of myelodysplastic syndrome were seen. Seven of the acute leukaemias occurred among 37 patients treated with hydroxyurea only (three in patients with polycythaemia vera, one in a patient with essential thrombocythaemia and three in patients with myelofibrosis), and two of the cases of acute leukaemia and one case of myelodysplastic syndrome occurred among 13 patients treated with alkylating agents prior to entrance into the study, yielding transformation frequencies of [19%] with hydroxyurea and [23%] with previous treatment.

Forty-two patients with polycythaemia vera (16 of whom were treated with hydroxyurea only), 15 with essential thrombocythaemia, six with myelofibrosis with myeloid metaplasia and one with an unclassified myeloproliferative disorder seen at a medical centre in Illinois, USA, during 1993–95 were evaluated for subsequent development of acute leukaemia or myelodysplastic syndrome (Nand *et al*., 1996). At the date of entry into the study, the patients had survived for a median of 5.2 years (range, four months to 20 years) since diagnosis of their chronic myeloproliferative disease. Five (7.8%) of the 64 cases transformed into acute leukaemia or myelodysplastic syndrome; none of these were in the 11 patients who had received no treatment or aspirin, two (11%) occurred in 18 patients treated with phlebotomy alone, two (8%) in 25 patients who had received hydroxyurea and one (10%) in 10 patients who had received only other immunosuppressive therapy. [The Working Group noted that the study group was composed of prevalent cases of chronic myeloproliferative disease only and that the follow-up period for most patients must have been very short.]

2.2.4 *Sickle-cell anaemia*

In order to test the efficacy of hydroxyurea in reducing the frequency of painful crises in adults with sickle-cell anaemia, Charache *et al*. (1995) conducted a randomized, placebo-controlled clinical trial. Of 299 such patients, 152 were assigned to hydroxyurea, while 147 were given placebo. Because of the beneficial effects observed, the trial was stopped after a median of 21 months (range, 14–24 months). At that time, no cases of leukaemia or other neoplastic disorders were seen.

To assess the safety and efficacy of hydroxyurea for the treatment of severe sickle-cell anaemia in children, Scott *et al*. (1996) conducted a small prospective study of 15 patients in one treatment centre in Illinois, USA. Thirteen patients in whom sickle-cell anaemia had been diagnosed in 1992–95 received hydroxyurea for a median of two years (range, 0.5–3.3 years), during which time no cases of acute leukaemia or other malignancies were seen.

2.2.5 *Congenital heart disease*

Sixty-four patients ranging in age from 8 to 47 years with inoperable cyanotic congenital heart disease were included in a prospective study in a treatment centre in France (Triadou *et al.*, 1994). The patients received hydroxyurea at an initial dose of 10 mg/kg bw per day, which was adapted according to haematological tolerance and continued over a period ranging from [two to 15 years] (mean, approximately five years). No cases of acute leukaemia or other malignancies were seen.

3. Studies of Cancer in Experimental Animals

The Working Group was aware of early studies in mice (Bhide & Sirsat, 1973) and in rats (Philips & Sternberg, 1975), which were considered inadequate for evaluation.

3.1 Intraperitoneal administration

Groups of 50 mice of each sex of the XVII/G strain were treated intraperitoneally with hydroxyurea [purity not specified] starting at two days of age and then at weekly intervals for one year. The doses per mouse were: 1 mg at two days of age, 3 mg at eight days, 5 mg at 15 days and 10 mg from 30 days to one year of age. One group of 50 mice was kept untreated as controls. The incidences of pulmonary tumours were 30/50 (60%) in control and 16/35 (46%) in treated mice. In a positive control group treated with urethane, 28/30 (93%) of mice had lung tumours (Muranyi-Kovacs & Rudali, 1972).

3.2 Administration with known carcinogens

Groups of 40 female Swiss mice, six to seven weeks of age, received dermal applications of 5 μg of 7,12-dimethylbenz[*a*]anthracene followed four weeks later by treatment with 1% croton oil for 14 weeks. Hydroxyurea at a dose of 500 mg/kg bw was injected intraperitoneally once at 24 h or twice at 24 and 48 h after the first painting with croton oil. Treatment with two doses of hydroxyurea significantly reduced the incidence of skin papillomas when compared with 7,12-dimethylbenz[*a*]anthracene and croton oil treatment alone (Chan *et al.*, 1970).

Groups of 16 male and 16 female hairless (hr/hr) Oslo mice were given an intraperitoneal injection of 0 or 5 mg hydroxyurea in 0.5 mL distilled water 30 min before dermal application of 2 mg of *N*-methyl-*N*-nitrosourea (MNU). Hydroxyurea enhanced the production of skin tumours by MNU from about 50% to 80%, this effect being attributed to inhibition of DNA synthesis (Iversen, 1982a). No such effect was observed when hydroxyurea was administered simultaneously with or after dermal application of 1 mg of MNU (Iversen, 1982b).

Groups of 36–43 female Wistar rats, weighing about 200 g, were given intraperitoneal injections of hydroxyurea in 23 fractionated consecutive doses of 0.1 mg/kg bw each shortly before and during maximal urothelial cell proliferation (33–55 h after partial cystectomy) produced by MNU administered as a single intravesicular pulse dose of 5 mg/kg bw during the various cell cycle phases. Hydroxyurea inhibited MNU-induced urothelial tumour development, and the degree of this inhibition depended on the cell cycle phase during which MNU was instilled. The numbers of rats with urothelial bladder tumours were: 14/43 in the control group (G_0 phase) and 7/37, 4/43 ($p < 0.02$), 10/46, 10/38, 9/36 and 12/40 in groups receiving MNU during the late G_1, early and late S, G_2+M, and early and late postmitotic phases, respectively (Kunze *et al.*,1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Although hydroxyurea has been in clinical use for 30 years, the pharmacokinetics of the compound has been extensively studied only recently. Two useful reviews have been published (Donehower, 1992; Gwilt & Tracewell, 1998), but the best of the limited data available come from the study of Rodriguez *et al.* (1998). These investigators gave 2 g of hydroxyurea either orally or by intravenous infusion over 30 min in a cross-over design to 29 patients with advanced cancers. They demonstrated clearly that oral and intravenous administration have essentially identical kinetics except for a 19.5% greater maximum plasma concentration (C_{max}) after intravenous dosing; the lag time of the peak after oral dosing was 0.22 h. Hydroxyurea is essentially completely absorbed from the human gastrointestinal tract, with a narrow range between subjects. The half-time of hydroxyurea is short, with an initial half-time of 0.63 h after intravenous administration and 1.78 h after oral administration and a terminal half-time of 3.32 h after oral administration and 3.39 h after intravenous administration. The clearance of hydroxyurea given orally or intravenously is identical and rapid, at 76 mL/min per m^2, with a mean distribution volume of 19.7 L/m^2. In this study, slightly more than one-third of the

administered dose was recovered in the urine. The 2-g dose resulted in a mean C_{max} of 794 μmol/L after oral administration and 1000 μmol/L after intravenous administration and a mean integrated area under the curve of concentration–time (AUC) of 3600 μmol/L per h after intravenous and 3900 μmol/L per h after oral administration.

Belt *et al*. (1980) compared oral and intravenous administration of escalating doses of hydroxyurea to patients with advanced malignancies. The maximal tolerated dose was 800 mg/m^2 every 4 h by oral administration and 3.0 mg/m^2 per min when given intravenously as a continuous 72-h infusion. After oral administration of doses of 500 or 800 mg/m^2 every 4 h, the peak concentration in plasma ranged from 5.4 to 24.8×10^{-4} mol/L. The time to attain the peak concentrations was 30–120 min. Two- to threefold variations among patients in the C_{max} after oral dosing were found. The C_{max} values for continuous intravenous infusion of doses of 2.0–3.5 mg/min per m^2 were $5.0–11.5 \times 10^{-4}$ mol/L. The plasma half-times of hydroxyurea in patients given single oral doses of 400–1200 mg/m^2 ranged from 132 to 279 min. In pleural fluid samples obtained from two patients, the concentrations paralleled those found in plasma. The mean half-time after discontinuation of intravenous infusion was about 250 min.

Hydroxyurea enters the cerebrospinal fluid, ascites fluid and serum (Beckloff *et al*., 1965) and breast milk (Sylvester *et al.*, 1987).

Villani *et al*. (1996) studied nine HIV-infected patients receiving hydroxyurea at 500 mg twice per day orally with or without zidovudine. The C_{max} was 0.135 mmol/L, and the C_{min} was 0.0085 mmol/L serum. The rate of clearance was 0.18 L/h per kg bw [12.6 L/h], with a half-time of 2.5 ± 0.5 h. The time to maximum clearance was approximately 0.9 h, and the bioavailability was good.

The maximum tolerated dose in a study of patients with chronic myeloid leukaemia in accelerated phase or blast crisis was 27 g/m^2 when given as a 24-h intravenous infusion (Gandhi *et al*., 1998). Intravenous doses of 8–40 g/m^2 resulted in plasma concentrations of 0.9–6.4 mmol/L with a half-time of approximately 3.5 h. A steady state was reached in all patients by 6 h. In this study, the dATP levels in peripheral blast cells decreased by 57%, but DNA synthesis decreased by 80–90%. The concentrations of the other deoxynucleotides were not affected.

About 30–60% of an orally administered dose of hydroxyurea is excreted unchanged by the kidneys (Donehower, 1992), although about 35% is generally excreted (Rodriguez *et al.*, 1998).

Andrae (1984) implicated a cytochrome P450-dependent process in metabolic activation of hydroxyurea which increases its potential for genetic damage. Hydrogen peroxide was reported to be a toxic metabolic product of hydroxyurea (Andrae & Greim, 1979). DeSesso *et al.* (1994) found that D-mannitol, a scavenger of free radicals, decreases the genotoxic effect of hydroxyurea. Sato *et al*. (1997) described pathways for the generation of nitric oxide from hydroxyurea via copper-catalysed peroxidation. Hepatic and renal conversion of hydroxyurea by a cytochrome c-dependent pathway to urea may account for 30–50% of administered doses. Urease may degrade hydroxyurea

to produce hydroxylamine and ultimately acetohydroxamic acid (Gwilt & Tracewell, 1998).

Both high and low doses show log-linear excretion kinetics, reflecting the predominance of renal mechanisms. The excretion of doses of 10–35 mg/kg bw diverges from linearity, probably because of an increasingly important saturable non-renal metabolic pathway (Villani *et al.*, 1996; Luzzati *et al.*, 1998).

4.1.2 *Experimental systems*

In contrast to the situation for humans, little information is available on the pharmacokinetics of hydroxyurea in animals, despite its wide use as a model teratogen and to synchronize the cell cycle in cell cultures. The lack of data may be due to the lack of a suitably sensitive assay during the early development of the drug (Donehower, 1992).

Van den Berg *et al*. (1994) gave nude mice doses of 0–200 mg/kg bw hydroxyurea by intraperitoneal injection and found a plasma concentration of 159 μmol/L within a half-time of only 11 min.

Wilson *et al.* (1975) found that the half-time of hydroxyurea in rats given 137 mg/kg bw per day intraperitoneally on days 9–12 of gestation was 15 min in the dams and 85 min in the embryos. In rhesus monkeys given 100 mg/kg bw per day intravenously on days 23–32 of gestation, the half-time was 120 min after the last injection in the mothers and 265 min in their fetuses.

4.2 Toxic effects

4.2.1 *Humans*

The major dose-limiting (and dose-related) toxic effects of hydroxyurea are granulocytopenia, which resolves relatively rapidly after withdrawal of the drug (Belt *et al.*, 1980), and myelosuppression, seen in advanced chronic myeloid leukaemia (Gandhi *et al.*, 1998). Drug-induced dermopathy with characteristics of dermatomyositis have been reported (Richard *et al.*, 1989; Velez *et al.*, 1998) as well as hyperpigmentation of the nails (Gropper *et al.*, 1993; de Montalembert *et al.*, 1997) and leg ulcers (Cox *et al.*, 1997; Weinlich *et al.*, 1998), although one large study of patients with sickle-cell anaemia found an equal rate of leg ulcers in patients given the placebo (Charache *et al*., 1996). In two- (Charache *et al*., 1996) and three-year (de Montalembert *et al.*, 1997) follow-up studies of patients with sickle-cell anaemia treated continuously with hydroxyurea, no serious side-effects other than mild neutropenia were observed, and this did not limit treatment.

When very high doses are given intravenously, dose-related mucositis is seen (Gandhi *et al*., 1998), but neutropenia is usually the treatment-limiting side-effect.

4.2.2 *Experimental systems*

No formal toxicological studies on hydroxyurea in animals were available to the Working Group.

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Information on the use of hydroxyurea in pregnancy is limited to a few case reports. Five case reports involved exposure to hydroxyurea for periods ranging from seven months to four years before pregnancy and throughout gestation at doses of 0.5–3.0 g daily. One woman developed eclampsia at 26 weeks and delivered a stillborn but phenotypically normal infant (Delmer *et al.*, 1992). The other four pregnancies ended in four normal, healthy infants at 36–40 weeks of gestation, with normal blood counts and normal postnatal development up to a maximum of 32 months (Patel *et al*., 1991; Delmer *et al*., 1992; Tertian *et al*., 1992; Jackson *et al*., 1993). Three other cases have been reported: one woman received a single dose of 8 g of hydroxyurea at about 12 weeks of pregnancy and had an elective termination four weeks later of an apparently normal fetus (Doney *et al*., 1979). Another woman was treated with an unspecified dose of hydroxyurea for six months before pregnancy and from mid-second trimester to near term, and delivered a healthy infant who developed normally during one year of follow-up (Fitzgerald & McCann, 1993). The third case involved a woman who had been treated with an unspecified dose of hydroxyurea two years before conception. She delivered a normal infant, who had normal physical and mental development at seven years of age (Pajor *et al*., 1991). [The Working Group noted that the doses of hydroxyurea used clinically are about one-fifth to one-tenth of the teratogenic dose in rodents.]

4.3.2 *Experimental systems*

Studies on the teratogenicity of hydroxyurea in chicks, mice, rats, rabbits, cats and monkeys have been published since the original reports by Murphy and Chaube (1964) and Chaube and Murphy (1966), who showed that a single intraperitoneal dose of 250 mg/kg bw or more given to Wistar rats on one of days 9–12 of gestation produced a high proportion of fetuses with multiple gross malformations of the central nervous system, palate and skeleton.

Pregnant NMRI mice injected intraperitoneally on day 10 of gestation with 500 mg/kg bw hydroxyurea showed marked necrosis of the neuroepithelium of the spinal cord 4 h after injection. The cytotoxicity could be partially prevented by simultaneous injection of 700 mg/kg bw deoxycytidine monophosphate (Herken, 1984) and completely prevented by simultaneous injection of 1 mg/kg bw colchicine (Herken, 1985). These results suggest that the action of hydroxyurea is dependent both on DNA synthesis and on the cytoskeleton.

Pregnant Wistar-derived rats were dosed intraperitoneally on day 12 of gestation with 250, 500, 750 or 1000 mg/kg bw hydroxyurea, and the fetuses were examined on day 20. A dose-related increase in the frequency of multiple malformations of the viscera and skeleton and reduced fetal weight were observed at doses ≥ 500 mg/kg bw, but embryolethality was seen only at 1000 mg/kg bw. Hydroxyurea was shown to pass into the embryo and to persist there longer than in maternal blood. DNA synthesis, as measured by thymidine incorporation into the embryo, was depressed markedly by doses ≥ 500 mg/kg bw, and marked cytotoxicity was also observed (Scott *et al*., 1971).

Studies from the same laboratory with the same strain of rat showed that intraperitoneal injection of 375 or 500 mg/kg bw hydroxyurea on day 12 of pregnancy produced microscopic evidence of cytotoxicity in the neural tube, but no malformations were observed when the dams were allowed to deliver their pups at term. Nevertheless, observation of the offspring at 30–50 days of age showed locomotor and behavioural deficits at both doses (Butcher *et al*., 1973). Further studies from the same laboratory with the same strain of rat showed that teratogenic and embryolethal effects could be induced by a dose as low as 137 mg/kg bw, but not by 100 mg/kg bw, administered intraperitoneally on days 9–12 of gestation (Wilson *et al*., 1975). Behavioural effects were also observed in the offspring of Sprague-Dawley dams treated with a single intraperitoneal dose of 150 mg/kg bw hydroxyurea on various days of pregnancy (Brunner *et al*., 1978). The wide range of malformations induced in rats by hydroxyurea has led to its use as a positive control substance in standard testing for both teratogenicity (Aliverti *et al*., 1980; Price *et al*., 1985) and developmental toxicity (postnatal behaviour) (Vorhees *et al*., 1979, 1983). Comparisons of the teratogenic responses in various stocks and strains of rats showed differences in the type of malformation and the time of sensitivity in two stocks of Wistar rats (Barr & Beaudoin, 1981) and in Wistar and Fischer 344 rats (DePass & Weaver, 1982).

A group of 27 pregnant golden hamsters received an intravenous injection of 50 mg/kg bw hydroxyurea on day 8 of pregnancy. The embryos were examined for external malformations only. A high rate of fetal death and malformations, especially of the central nervous system, was observed (Ferm, 1966).

The teratogenicity of hydroxyurea in pregnant New Zealand white rabbits was demonstrated by subcutaneous injection of 750 mg/kg bw once on day 12 of gestation, with embryo and fetal examination 15 min to 32 h later by histology and on day 29 for malformations. Treatment produced marked cytotoxicity and a high percentage of resorptions (61%), reduced fetal weight and malformations in all surviving fetuses affecting most organ systems and the skeleton, as observed in rats (DeSesso & Jordan, 1977; DeSesso, 1981a). The mechanism by which hydroxyurea produces its teratogenic action was investigated in detail by DeSesso and his co-workers, who showed in rabbits that hydroxyurea is not only cytotoxic and inhibits DNA synthesis but also causes a very rapid, marked reduction in uterine–placental blood flow, which may be responsible for some of the teratogenic effects (Millicovsky *et al*., 1981). In addition, the teratogenic effects can be inhibited by simultaneous administration of the anti-oxidant

propyl gallate, which reduces the cytotoxicity (DeSesso, 1981b). This activity occurs within the embryo and is independent of the inhibition of DNA synthesis (DeSesso & Goeringer, 1990). Inhibition of the cytotoxicity and teratogenicity of hydroxyurea by D-mannitol, a potent scavenger of hydroxyl free radicals, suggests that these radicals are the proximate cytotoxins and teratogens (DeSesso *et al*., 1994).

Groups of 17 mated cats of European and Persian breeds were dosed orally with 50 or 100 mg/kg bw hydroxyurea on days 10–22 of gestation, and the fetuses were examined on day 43. At 50 mg/kg bw, fetal weight and survival were not affected, but a high proportion of the fetuses were malformed, with a wide range of malformations similar to those seen in other species. At 100 mg/kg bw, a large proportion of the cats were not pregnant, but maternal and fetal weights were reduced, the frequency of resorptions increased and one of two live fetuses was malformed (cyclopia) (Khera, 1979).

Of 22 pregnant female rhesus monkeys (*Macaca mulatta*) dosed intravenously with 50–500 mg/kg bw hydroxyurea for various times between days 18 and 45 of gestation, eight aborted or had intrauterine deaths; 10 had fetuses with multiple malformations mostly of the axial skeleton, but also genitourinary, cardiac, brain, eye and intestinal defects; and the infants of three were growth retarded and one was normal (Theisen *et al*., 1973; Wilson, 1974; Wilson *et al.*, 1975). [The Working Group noted that little detailed information is given in these reports.]

The teratogenicity of hydroxyurea was compared in mouse embryos *in vivo* and *in vitro*, to study the effects of varying the concentration of drug and the duration of exposure. Mated ICR mice were injected intraperitoneally with 300 mg/kg bw hydroxyurea on day 9 of pregnancy (vaginal plug = day 1), and the embryos were removed 48 h later for examination for malformations and for protein content. The embryos of untreated mice were removed on day 9 and cultured *in vitro* in various concentrations of hydroxyurea for various lengths of time, followed by culture in drug-free medium up to 48 h. *In vivo*, 45% of the embryos showed malformations, including exencephaly and phocomelia, and the peak plasma concentration of hydroxyurea was 311 ± 22 μg/mL 7 min after injection, with a half-time of 30 min. Culture *in vitro* with hydroxyurea at 300 μg/mL for 30 min resulted in malformations in 41% of the embryos that were similar to those found *in vivo*. Culture at a concentration of 500 μg/mL for 30 min or at 250 μg/mL for 1 h resulted in 100% malformed embryos, but culture at 125 μg/mL for 1 h resulted in no malformations (Warner *et al*., 1983). Culture of 10-day CD rat embryos and eight-day CD-1 mouse embryos with 300 μg/mL hydroxyurea for 1 h followed by 43 h in drug-free medium resulted in impaired development, and the embryos had reduced DNA and protein contents. Addition of various concentrations of dAMP to the culture medium did not inhibit the action of hydroxyurea, and addition of dCMP had minimal inhibitory activity. Hydroxyurea decreased all nucleotide pools, and addition of dAMP increased the pools but not to control levels (Hansen *et al*., 1995).

Malformations were also produced in chicks injected *in ovo* on day 4 with 800 μg of hydroxyurea (Iwama *et al*., 1983).

Seven groups of at least six male C57BL/6J×C3H/HeJ F_1 mice were injected when 13–15 weeks of age with 0, 25, 50, 100, 200, 400 or 500 mg/kg bw hydroxyurea intraperitoneally daily for five consecutive days. The epididymides and testes were examined eight and 29 days after the last injection. Body weight was not affected in any of the animals, but the testis weight was reduced in a dose-related manner at all doses except the lowest. A dose-related reduction in DNA synthesis was seen, resulting in depletion of pachytene spermatocytes and a consequent reduction in later cell stages and spermiogenesis. Spermatogonial stem cells were not affected, and showed repopulation of cell stages with normal differentiation kinetics (Evenson & Jost, 1993). Similar results were reported in B6C3/F_1/BOM M mice aged six to eight weeks injected intraperitoneally with 200 mg/kg bw hydroxyurea for five days (Wiger *et al*., 1995).

4.4 Genetic and related effects

4.4.1 *Humans*

In studies of genetic alterations in leukaemic cells of patients treated with hydroxyurea, a statistically non-significant association was seen between treatment with hydroxyurea alone or in combination and the occurrence of leukaemia and myelodysplastic syndrome characterized by abnormalities of chromosome 17 in patients with essential thrombocythaemia [p = 0.11, Fisher's exact test]. As discussed in section 2, Sterkers *et al.* (1998) monitored the occurrence of acute leukaemia and myelodysplastic syndrome in 251 patients with essential thrombocythemia who were treated with hydroxyurea. The findings in the leukaemic cells are summarized in Table 2. In seven cases of leukaemia treated with hydroxyurea, including three given the drug alone, there were rearrangements of chromosome 17, including unbalanced translocations, partial or complete deletions and isochromosome 17q, which resulted in 17p deletion in the leukaemic cells. *P53* mutation was observed in six cases, including two treated with hydroxyurea alone. The authors suggested that the molecular characteristics of these

Table 2. Karyotypic findings in the bone marrow of patients with essential thrombocythaemia treated with hydroxyurea

Treatment	Leukaemia or myelodysplastic syndrome			No leukaemia	Total no. of patients
	17p deletion	No 17p deletion	Karyotype unavailable		
Hydroxyurea	3	2	2	194	201
Hydroxyurea plus other agents	4	1	2	43	50
No hydroxyurea	0	3	0	104	107

Derived from Sterkers *et al*. (1998)

leukaemias are consistent with 17p$^-$ syndrome and that prolonged use of hydroxyurea in patients with essential thrombocythaemia may lead to acute myeloid leukaemia and myelodysplastic syndrome with loss of chromosome 17p material and *P53* mutation. A review of the literature by these authors revealed similar 17p deletions in four of 11 patients treated for essential thrombocythaemia with hydroxyurea alone but in only one of 24 patients who did not receive this treatment. Tefferi (1998) cautioned, however, that the results of bone-marrow and cytogenetic investigations before treatment were not available for some of the patients. Monosomy 17 was also observed in complex karyotypes in two of three cases of leukaemia reported by Liozon *et al.* (1997) among 58 patients with essential thrombocythaemia treated with hydroxyurea; in the third case, which was chronic myelomonocytic leukaemia, the karyotype was normal.

Quesnel *et al.* (1993) identified the t(8;21) translocation in a case of leukaemia in which essential thrombocythaemia had been treated with hydroxyurea alone. The t(8;21) is associated with the French–American–British M2 (acute myeloblastic) subtype of de-novo and treatment-related acute myeloid leukaemia. The complex karyotype, which also contained monosomy 17, was 46, XX[3]/47, XX, +8 [2]/43–44,XX,der(7)t(7;dup5), t(8;21)(q22;q22), –16,–17,t(18;?)(q;?)[10].

Ören *et al.* (1999) described a case of acute promyelocytic leukaemia with i(17q) after treatment of Philadelphia chromosome-positive chronic myeloid leukaemia with combination therapy including hydroxyurea, but i(17q) may occur in chronic myeloid leukaemia in blast crisis.

Diverse chromosomal aberrations have been seen in human bone-marrow cells after hydroxyurea treatment. Diez-Martin *et al.* (1991) reviewed studies of the chromosomes of 104 patients at various stages of polycythaemia vera. The bone-marrow cells of five of six patients treated with hydroxyurea alone had abnormalities, including an unbalanced t(1;7)(p11;p11), which can be associated with treatment-related myelodysplastic syndrome, but this abnormality may occur without prior treatment. Cytogenetic analyses in these five patients were performed only on bone-marrow samples obtained after treatment. One each of the other four abnormal marrows had t(8;13)(p21;q12), +9, del(6)(q13q21) and t(1;?)(q12;?). Furthermore, the authors observed several de-novo abnormalities in untreated patients which they related to the disease itself rather than to the therapy, including +9, +8 and 20q–, and suggested that the 13q– abnormality is related to disease progression.

Löfvenberg *et al.* (1990) examined 81 hydroxyurea-treated patients with Philadelphia chromosome-negative chronic myeloproliferative disorders, comprising 35 with polycythaemia vera, 32 with essential thrombocythaemia, 12 with myelofibrosis and two with myeloproliferative syndromes. Only three had received prior therapy with alkylating agents or radioactive phosphorus. Four of the 81 developed acute myeloid leukaemia or myelodysplastic syndrome. Five of 53 evaluable patients (9%) had clonal cytogenetic abnormalities involving chromosomes 1, 9, 20 and 21 before treatment, and 15% had these abnormalities at follow-up, during or after hydroxyurea treatment. Treatment was thus associated with a low frequency of cytogenetic

abnormalities in a heterogeneous population, and the abnormalities observed before and after treatment were similar.

The series later reported on by Weinfeld *et al.* (1994) included 30 patients with polycythaemia vera, 10 with essential thrombocythaemia and 10 with myelofibrosis who were treated with hydroxyurea. Acute leukaemia developed in nine patients and myelodysplastic syndrome in one; seven of the leukaemia patients had been treated with hydroxyurea alone. The duration of therapy for patients who developed leukaemia or myelodysplastic syndrome was 5–111 months. Seven of 19 previously untreated patients with initially normal karyotypes treated with hydroxyurea alone developed clonal chromosomal abnormalities during therapy (37%).

Davidovitz *et al.* (1998) observed evolution of polycythaemia vera to myelofibrosis with a t(1;20)(q32;q13.3) in a patient who received chronic low-dose hydroxyurea. The t(1;20) affected the same region of chromosome 20 as the 20q– abnormality; it could not be determined whether the translocation was related to the treatment. Furgerson *et al.* (1996) described a patient in whom essential thrombocythaemia evolved to acute myeloid leukaemia after hydroxyurea treatment. The karyotype was normal at the time of diagnosis of essential thrombocythaemia but revealed del(5)(q23), del(7)(q31), inv(16)(p13;q22),+8 when acute myeloid leukaemia emerged.

4.4.2 *Experimental systems*

Early studies on the mutagenicity of hydroxyurea were summarized by Timson (1975). Reviews on the mutagenicity of anticancer drugs in general, including hydroxyurea, were provided by Ferguson (1995) and Jackson *et al*. (1996). Ferguson and Denny (1995) commented on some practical issues in testing antimetabolites, which may limit the usefulness (and meaning) of some types of in-vitro assays.

The results of tests for genotoxicity with hydroxyurea are summarized in Table 3.

Hydroxyurea was inactive as either a frameshift or base-pair substitution mutagen in *Salmonella typhimurium* strains TA1537, TA1535, TA98 and TA100, and addition of an exogenous metabolic activation system did not affect these results. Hydroxyurea induced SOS repair in *Escherichia coli* K12 cells. In various *Saccharomyces cerevisiae* strains, hydroxyurea induced mitotic crossing over, mitotic gene conversion, intrachromosomal recombination and aneuploidy, but not 'petite' mutations. It also increased the frequency of ultraviolet-induced mitotic gene conversion and induced recombination in dividing but not G1 or G2 arrested cells of the RS112 strain of yeast. In meiotic yeast cells, hydroxyurea increased the frequency of meiotic recombination.

Hydroxyurea is a clastogen in mammalian cells *in vitro* and *in vivo*.

Hydroxyurea caused chromosomal aberrations in cultured Chinese hamster cells, in mouse cells and in various human cell lines. Karon and Benedict (1972) found that hydroxyurea induced chromosomal aberrations when given during S phase but not when given during G2 phase. It did not induce micronuclei in human peripheral blood lymphocytes but increased the frequency of sister chromatid exchange and of gene

Table 3. Genetic and related effects of hydroxyurea

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli K12, SOS repair response	+	NT	7600	Barbé *et al*. (1987)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Haworth *et al*. (1983)
Saccharomyces cerevisiae D5, petite mutations	–	NT	10 000	Ferguson & Turner (1988a)
Saccharomyces cerevisiae D5, mitotic crossing-over	+	NT	2400	Ferguson & Turner (1988b)
Saccharomyces cerevisiae D61.M, mitotic gene conversion	+	NT	7600	Mayer *et al*. (1986)
Saccharomyces cerevisiae D61.M, aneuploidy	+	NT	7600	Mayer *et al*. (1986)
Saccharomyces cerevisiae RS112, intrachromosomal recombination	+	NT	380	Galli & Schiestl (1996)
Saccharomyces cerevisiae SBTD and D7, increased frequency of ultraviolet-induced mitotic gene conversion	+	NT	2280	Zaborowska *et al*. (1983)
Saccharomyces cerevisiae 419 and 580, meiotic recombination	+	NT	3040	Simchen *et al*. (1976)
Drosophila melanogaster larvae, chromosomal aberrations, mitotic brain ganglion cells *in vitro*	+	NT	7.6	Banga *et al.* (1986)
Drosophila melanogaster larvae, chromosomal aberrations, brain ganglia *in vivo*	+	NT	6080	Banga *et al.* (1986)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	760	Rossberger & Andrae (1985)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	3	Amacher & Turner (1987)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	0.7	Wangenheim & Bolcsfoldi (1988)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+[c]	+[c]	20	Sofuni *et al*. (1996)
Sister chromatid exchange, Chinese hamster lung V79-4 cells *in vitro*	–	NT	0.5	Popescu *et al*. (1977)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster lung V79 B-1 cells *in vitro*	+	NT	7.6	Ishii & Bender (1980)
Sister chromatid exchange, Chinese hamster lung V79 and ovary cells *in vitro*	+	NT	76	Mehnert *et al.* (1984)
Sister chromatid exchange, Chinese hamster ovary CHO-B11 cells *in vitro*	+	NT	23	Hahn *et al.* (1986)
Sister chromatid exchange, mouse lymphoma L5178Y Jsens and C3 cells *in vitro*	+	NT	76	Hill & Schimke (1985)
Sister chromatic exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	76	Tohda & Oikawa (1990)
Chromosomal aberrations, Chinese hamster lung V79-4 cells *in vitro*	+	NT	0.5	Popescu *et al.* (1977)
Chromosomal aberrations, Chinese hamster ovary CHO-B11 cells *in vitro*	+	NT	23	Hahn *et al.* (1986)
Chromosomal aberrations, Chinese hamster Don-C cells *in vitro* (S phase)	+	NT	100	Karon & Benedict (1972)
Chromosomal aberrations, mouse lymphoma L5178Y Jsens and C3 cells *in vitro*	+	NT	76	Hill & Schimke (1985)
Cell transformation, cultures of embryonic cells from BN/a mice, with confirmation in newborn mice	+	NT	0.76	Chlopkiewicz & Koriorowska (1983)
Cell transformation, cultures of embryonic cells from DBA/2 and Swiss mice	–	NT	Not reported	Chlopkiewicz & Koriorowska (1983)
Cell transformation, BALB/c 3T3 cells mouse cells	–	NT	7.6	Chlopkiewicz & Koriorowska (1983)
DNA single-strand breaks, Ehrlich ascites tumour cells *in vitro*	+	NT	38	Li & Kaminskas (1987)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single-strand breaks, human T lymphoma CCRF-CEM cells *in vitro*	+	NT	4.6	Skog *et al*. (1992)
Mutation, human T-lymphoblast cell line, *HPRT* locus *in vitro*	–	NT	19	Mattano *et al*. (1990)
Micronucleus formation, human primary lymphocytes *in vitro*	–	NT	1520	Fenech *et al*. (1994)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	150	Kihlman & Andersson (1985)
Chromosomal aberrations, human Hep.2 cell line *in vitro*	+	NT	190	Strauss *et al*. (1972)
Chromosomal aberrations, human B lymphoblast TK6, WI-L2-NS and WTK1 cell lines *in vitro*	+	NT	76	Greenwood *et al*. (1998)
Specific locus mutation, (101/H male × C3H/HeH female)F_1 mice	–		500 ip × 2	Cattanach *et al*. (1989)
Micronucleus formation, bone-marrow cells, male NMRI mice *in vivo*	+		400 ip × 1	Hart & Hartley-Asp (1983)
Micronucleus formation, bone-marrow cells, female C57BL/6 × C3H/He hybrid mice *in vivo*	–		250 ip × 5	Bruce & Heddle (1979)
Chromosomal aberrations, spermatogonial cells, male Swiss mice	–		500 ip × 1	van Buul & Bootsma (1994)
Dominant lethal assay, male ICR/Ha Swiss mice	–		1000 ip × 1	Epstein *et al*. (1972)
Sperm morphology, C57BL/6 × C3H/He hybrid mice *in vivo*	+		250 ip × 5	Bruce & Heddle (1979)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitoneally
[c] Same result in two laboratories

amplification in L5178Y mouse lymphoma cells. Hydroxyurea induced sister chromatid exchange in various Chinese hamster cell lines *in vitro*. It caused DNA strand breaks in Ehrlich ascites tumour cells and in human T lymphoma cells *in vitro*.

It did not induce mutations at the *HPRT* locus in a cultured human T-lymphocyte cell line at doses of 50–250 μmol/L, which had substantial effects on DNA synthesis, but induced mutants at the *Tk* locus in L5178Y cells.

Hydroxyurea caused cell transformation in mass cultures of embryonic cells from BN/a, mice, but not in cultures derived from two other strains of mice—DBA/2 and Swiss, nor in BALB/c 3T3 cells. Although hydroxyurea alone did not induce morphological transformation in Syrian hamster embryo cells, the cell cycle arrest caused by the drug led to enhancement of cell transformation by bromodeoxyuridine (Tsutsui *et al.*, 1979). Hydroxyurea did not enhance metabolic cooperation between V79 cells (Toraason *et al.*, 1992).

Hydroxyurea treatment led to hypermethylation of DNA in hamster fibrosarcoma cells (Nyce *et al.*, 1986). In rats, this resulted in nitric oxide production (Jiang *et al.*, 1997) and induced a cytokine response (Navarra *et al.*, 1995, 1997). These effects may indicate an enhanced effect on chromosomal damage in certain situations *in vivo*. Hydroxyurea also induced DNA hypermethylation in normal human embryonic lung fibroblasts (WI-38) and their simian virus 40-transformed counterparts (SVWI-38) (De Haan & Parker, 1988).

Hydroxyurea induced micronuclei in the bone marrow of non-tumour-bearing male NMRI mice but did not induce micronucleated cells in female C57BL/6 × C3H/He hybrid mice, although it produced sperm abnormalities in male mice of this strain.

Although hydroxyurea is a mutagen in somatic cells, there is no evidence that it mutates germ cells. It did not cause dominant lethal mutation or specific locus mutation in mice. It did not induce chromosomal damage in spermatogonial cells of male Swiss mice, although it enhanced damage induced by X-rays.

Minford *et al*. (1984) showed that hydroxyurea at 0.1 mmol/L enhanced both DNA breakage and cytotoxicity caused by the intercalating DNA topoisomerase II inhibitor, amsacrine. Lambert *et al*. (1983) found similar results in relation to adriamycin. Palitti *et al*. (1984a) showed that treatment with hydroxyurea after mitomycin C enhanced the frequencies of chromosomal aberrations and sister chromatid exchange induced by mitomycin C alone in both Chinese hamster cells and human lymphocytes. Hydroxyurea had a synergistic effect on ultraviolet-induced sister chromatid exchange (Ishii & Bender, 1980) and enhanced X-radiation-induced damage in spermatogonial cells of Swiss mice (van Buul & Bootsma, 1994).

4.5 Mechanistic considerations

Hydroxyurea does not bind or bond to DNA but acts by inhibiting ribonucleotide reductase, which converts ribonucleoside diphosphates to deoxyribonucleotide diphosphates, the precursers for de-novo DNA synthesis. Hydroxyurea depletes intracellular

deoxyribonucleotide pools and is known and used as an inhibitor of DNA synthesis (Timson, 1975).

The differences in the results of various studies may depend on the exact cell culture conditions, especially in regard to the amounts of deoxyribonucleotides available. Hansen *et al*. (1995) found that they could partially attenuate the embryotoxic effects of hydroxyurea by providing additional deoxyribonucleotides.

In a number of experiments, hydroxyurea appeared to enhance the susceptibility of cells to mutagenesis by other agents (e.g. Palitti *et al*., 1983, 1984a,b; Ferguson, 1990; Jelmert *et al*., 1992). There are three possible reasons for this:

1. It halts the progression of cells in the late G1 phase of the cycle, allowing synchronization of the culture (e.g. Tsutsui *et al*., 1979). Thus, if cells are sensitive to a certain agent in a particular phase of the cell cycle, hydroxyurea may reveal this effect.
2. Although hydroxyurea inhibits normal DNA synthesis, it does not appear to inhibit unscheduled DNA synthesis at the same doses after treatment with various genotoxic agents, including X-rays (e.g. Painter & Cleaver, 1967). This justifies its inclusion in protocols of unscheduled DNA synthesis.
3. Prempree and Merz (1969) suggested that hydroxyurea could inhibit the repair of chromosomal breaks without itself inducing breaks.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hydroxyurea is a chemically simple antimetabolite that inhibits the enzyme ribonucleotide reductase. It has been in clinical use since the 1960s and is widely used for the treatment of severe sickle-cell disease, chronic myeloid leukaemia, myeloproliferative disorders such as polycythaemia vera and essential thrombocythaemia and, increasingly, in combination with didanosine in HIV infection. Hydroxyurea is sometimes used for the treatment of psoriasis and various solid tumours.

5.2 Human carcinogenicity data

The risk for leukaemia associated with administration of hydroxyurea in the treatment of chronic myeloproliferative disorders has been evaluated in a number of small cohort studies. Overall, 5–6% of patients developed either acute leukaemia or myelodysplastic syndrome subsequent to the start of hydroxyurea treatment. Large variation in the length of active follow-up was not taken into account in the analyses. The risk for leukaemia in patients with chronic myeloproliferative disorders who were not treated with hydroxyurea or other agents (e.g. polycythaemia vera patients treated with phlebotomy alone) was also increased in comparison with that of the general

population. The available data do not allow a conclusion about whether the occurrence of acute leukaemia and myelodysplastic syndrome in the hydroxyurea-treated patients represents progression of the myeloproliferative process or an effect of the treatment.

5.3 Animal carcinogenicity data

Hydroxyurea was tested in one experiment in mice by intraperitoneal administration beginning at two days of age. No increase in the incidence of tumours was reported. Hydroxyurea has also been tested in combination with other chemical carcinogens to assess the effect of inhibition of DNA synthesis on carcinogenesis. The experiments are inadequate to assess the carcinogenicity of hydroxyurea.

5.4 Other relevant data

Hydroxyurea is readily absorbed after oral administration. In one study, 35% of an administered dose was excreted unchanged in the urine of humans. Hydroxyurea is widely distributed in tissues. Its main toxic effect is neutropenia.

Hydroxyurea is teratogenic and causes postnatal behavioural deficits after prenatal exposure in all species of animals in which it has been tested. It has commonly been used as positive control substance in studies of developmental toxicity.

In one study of patients treated with hydroxyurea for essential thrombocythaemia who developed leukaemia, a statistically non-significant association was found with a 17p chromosomal deletion in leukaemic cells.

Hydroxyurea neither bonds chemically nor otherwise binds to DNA. Instead, it inhibits ribonucleotide reductase, which converts ribonucleoside diphosphates to deoxyribonucleotide diphosphate precursers for de-novo DNA synthesis. Hydroxyurea does not induce gene mutation in bacteria and does not cause mutation at the *Hprt* locus in mammalian cells. It causes chromosomal mutations and mutagenic effects at the *Tk* locus in mouse lymphoma cells. It is an effective recombinogen in yeast and induces sister chromatid exchange in mammalian cells. It also causes gene amplification in mammalian cells and may lead to transformation of some but not all cell lines. Although it has been reported to be ineffective in causing germ-cell mutation, it has not been extensively tested for that end-point.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of hydroxyurea.

There is *inadequate evidence* in experimental animals for the carcinogenicity of hydroxyurea.

Overall evaluation

Hydroxyurea is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Aliverti, V., Bonanomi, L. & Giavini, E. (1980) Hydroxyurea as a reference standard in teratological screening. Comparison of the embryotoxic and teratogenic effects following single intraperitoneal or repeated oral administrations to pregnant rats. *Arch. Toxicol.*, **Suppl. 4**, 239–247

Amacher, D.E. & Turner, G.N. (1987) The mutagenicity of 5-azacytidine and other inhibitors of replicative DNA synthesis in the L5178Y mouse lymphoma cell. *Mutat. Res.*, **176**, 123–131

American Hospital Formulary Service (1997) *AHFS Drug Information® 97*, Bethesda, MD, American Society of Health-System Pharmacists, pp. 766–770

Andrae, U. (1984) Evidence for the involvement of cytochrome P-450-dependent monooxygenase(s) in the formation of genotoxic metabolites from N-hydroxyurea. *Biochem. biophys. Res. Commun.*, **118**, 409–415

Andrae, U. & Greim, H. (1979) Induction of DNA repair replication by hydroxyurea in human lymphoblastoid cells mediated by liver microsomes and NADPH. *Biochem. biophys. Res. Commun.*, **87**, 50–58

van den Anker-Lugtenburg, P.J. & Sizoo, W. (1990) Myelodysplastic syndrome and secondary acute leukemia after treatment of essential thrombocythemia with hydroxyurea. *Am. J. Hematol.*, **33**, 152

Banga, S.S., Shenkar, R. & Boyd, J.B. (1986) Hypersensitivity of Drosophila *mei-41* mutants to hydroxyurea is associated with reduced mitotic chromosome stability. *Mutat. Res.*, **163**, 157–165

Barbé, J., Villaverde, A. & Guerrero, R. (1987) Induction of the SOS response by hydroxyurea in *Escherichia coli* K12. *Mutat. Res.*, **192**, 105–108

Barr, M., Jr & Beaudoin, A.B. (1981) An exploration of the role of hydroxyurea injection time in fetal growth and terartogenesis in rats. *Teratology*, **24**, 163–167

Beckloff, G.L., Lerner, H.J., Frost, D., Russo-Alesi, F.M. & Gitomer, S. (1965) Hydroxyurea in biologic fluids: Dose–concentration relationship. *Cancer Chemother. Rep.*, **48**, 57–58

Bellucci, S., Janvier, M., Tobelem, G., Flandrin, G., Charpak, Y., Berger, R. & Boiron, M. (1986) Essential thrombocythemias. Clinical evolutionary and biological data. *Cancer*, **58**, 2440–2447

Belt, R.J., Haas, C.D., Kennedy, J. & Taylor, S. (1980) Studies of hydroxyurea administered by continuous infusion: Toxicity, pharmacokinetics, and cell synchronization. *Cancer*, **46**, 455–462

Best, P.J.M. & Petitt, R.M. (1998) Multiple skin cancers associated with hydroxyurea therapy. *Mayo Clin. Proc.*, **73**, 961–963

Bhide, S.V. & Sirsat, M.V. (1973) Delayed effects of a single treatment of hydroxyurea to newborn mice. *Indian J. Cancer*, **10**, 26–30

Biron, F., Lucht, F., Peyramond, D., Fresard, A., Vallet, T., Nugier, F., Grange, J., Malley, S., Hamedi-Sangsari, F. & Vila, J. (1996) Pilot clinical trial of the combination of hydroxyurea and didanosine in HIV-1 infected individuals. *Antiviral Res.*, **29**, 111–113

Boyd, A.S. & Neldner, K.H. (1991) Hydroxyurea therapy. *J. am. Acad. Dermatol.*, **25**, 518–524

British Medical Association/Royal Pharmaceutical Society of Great Britain (1998) *British National Formulary*, No. 36, London, p. 377

British Pharmacopoeial Commission (1993) *British Pharmacopoeia 1993*, London, Her Majesty's Stationery Office, Vols I & II, pp. 343, 954, S68

Bruce, W.R. & Heddle, J.A. (1979) The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella*, and sperm abnormality assays. *Can. J. Genet. Cytol.*, **21**, 319–334

Brunner, R.L., McLean, M., Vorhees, C.V. & Butcher, R.E. (1978) A comparison of behavioral and anatomical measures of hydroxyurea induced abnormalities. *Teratology*, **18**, 379–384

Budavari, S., ed. (1996) *The Merck Index*, 12th Ed., Whitehouse Station, NJ, Merck & Co., p. 833

Butcher, R.E., Scott, W.J., Kazmaier, K. & Ritter, E.J. (1973) Postnatal effects in rats of prenatal treatment with hydroxyurea. *Teratology*, **7**, 161–165

van Buul, P.P. & Bootsma, A.L. (1994) The induction of chromosomal damage and cell killing in mouse spermatogonial stem cells following combined treatments with hydroxyurea, 3-aminobenzamide and X-rays. *Mutat. Res.*, **311**, 217–224

Callot-Mellot, C., Bodemer, C., Chosidow, O., Frances, C., Azgui, Z., Varet, B. & de Prost, Y. (1996) Cutaneous carcinoma during long-term hydroxyurea therapy: A report of 5 cases. *Arch. Dermatol.*, **132**, 1395–1397

Cattanach, B.M., Peters, J. & Rasberry, C. (1989) Induction of specific locus mutations in mouse spermatogonial stem cells by combined chemical X-ray treatments. *Mutat. Res.*, **212**, 91–101

Chan, P.C., Goldman, A. & Wynder, E.L. (1970) Hydroxyurea: Suppression of two-stage carcinogenesis in mouse skin. *Science*, **168**, 130–132

Charache, S., Terrin, M.L., Moore, R.D., Dover, G.J., Barton, F.B., Eckert, S.V., McMahon, R.P., Bonds, D.R. & the Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (1995) Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. *New Engl. J. Med.*, **332**, 1317–1322

Charache, S., Barton, F.B., Moore, R.D., Terrin, M.L., Steinberg, M.H., Dover, G.J., Ballas, S.K., McMahon, R.P., Castro, O., Orringer, E.P. & the Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (1996) Hydroxyurea and sickle cell anemia. Clinical utility of a myelosuppressive 'switching' agent. *Medicine*, **75**, 300–326

Chaube, S. & Murphy, M.L. (1966) The effects of hydroxyurea and related compounds on the rat fetus. *Cancer Res.*, **26**, 1448–1457

Chlopkiewicz, B. & Koziorowska, J.H. (1983) Transforming activities of methotrexate, hydroxyurea and 5-fluorouracil in different cell systems. *Neoplasma*, **30**, 295–302

CIS Information Services (1998) *Worldwide Bulk Drug Users Directory 1997/98 Edition*, Dallas, TX [CD-ROM]

Cox, C., Nowicky, D. & Young, R. (1997) Hydroxyurea-related ankle ulcers in patients with myeloproliferative disorders: A case report and review of the literature. *Ann. plast. Surg.*, **39**, 546–549

Currie, J.L., Blessing, J.A., Muss, H.B., Fowler, J., Berman, M. & Burke, T.W. (1996a) Combination chemotherapy with hydroxyurea, dacarbazine (DTIC), and etoposide in the treatment of uterine leiomyosarcoma: A Gynecologic Oncology Group study. *Gynecol. Oncol.*, **61**, 27–30

Currie, J.L., Blessing, J.A., McGehee, R., Soper, J.T. & Berman, M. (1996b) Phase II trial of hydroxyurea, dacarbazine (DTIC), and etoposide (VP-16) in mixed mesodermal tumors of the uterus: A Gynecologic Oncology Group study. *Gynecol. Oncol.*, **61**, 94–96

Davidovitz, Y., Lev, D., Ballin, A., Tsudik, A. & Meytes, D. (1998) Short communication. Translocation (1;20)(q32;q13.3) in myelofibrosis following polycythemia vera. *Cancer Genet. Cytogenet.*, **101**, 156–158

De Boer, R.J., Boucher, C.A. & Perelson, A.S. (1998) Target cell availability and the successful suppression of HIV by hydroxyurea and didanosine. *AIDS*, **12**, 1567–1570

De Haan, J.B. & Parker, M.I. (1988) Differential effects of DNA synthesis inhibitors on DNA methylation in normal and transformed cells. *Anticancer Res.*, **8**, 617–620

Delmer, A., Rio, B., Bauduer, F., Ajchenbaum, F., Marie, J.-P. & Zittoun, R. (1992) Pregnancy during myelosuppressive treatment for chronic myelogenous leukaemia (Letter to the Editor). *Br. J. Haematol.*, **82**, 783–784

DePass, L.R. & Weaver, E.V. (1982) Comparison of teratogenic effects of aspirin and hydroxyurea in the Fischer 344 and Wistar strains. *J. Toxicol. environ. Health*, **10**, 297–305

DeSesso, J.M. (1981a) Comparative ultrastructural alterations in rabbit limb-buds after a teratogenic dose of either hydroxyurea or methotrexate. *Teratology*, **23**, 197–215

DeSesso, J.M. (1981b) Amelioration of teratogenesis. I. Modification of hydroxyurea-induced teratogenesis by the antioxidant propyl gallate. *Teratology*, **24**, 19–35

DeSesso, J.M. & Goeringer, G.C. (1990) The nature of the embryo-protective interaction of propyl gallate with hydroxyurea. *Reprod. Toxicol.*, **4**, 145–152

DeSesso, J.M. & Jordan, R.L. (1977) Drug-induced limb dysplasias in fetal rabbits. *Teratology*, **15**, 199–211

DeSesso, J.M., Scialli, A.R. & Goeringer, G.C. (1994) D-Mannitol, a specific hydroxyl free radical scavenger, reduces the developmental toxicity of hydroxyurea in rabbits. *Teratology*, **49**, 248–259

De Simone, C., Guerriero, C., Guidi, B., Rotoli, M., Venier, A. & Tartaglione, R. (1998) Multiple squamous cell carcinomas of the skin during long-term treatment with hydroxyurea. *Eur. J. Dermatol.*, **8**, 114–115

Diez-Martin, J.L., Graham, D.L., Petitt, R.M. & Dewald, G.W. (1991) Chromosome studies in 104 patients with polycythemia vera. *Mayo Clin. Proc.*, **66**, 287–299

Disdier, P., Harle, J.R., Grob, J.J., Weiller-Merli, C., Magalon, G. & Weiller, P.J. (1991) Rapid development of multiple squamous-cell carcinomas during chronic granulocytic leukemia. *Dermatologica*, **183**, 47–48

Donehower, R.C. (1992) An overview of the clinical experience with hydroxyurea. *Semin. Oncol.*, **19** (Suppl. 9), 11–19

Doney, K.C., Kraemer, K.G. & Shepard, T.H. (1979) Combination chemotherapy for acute myelocytic leukemia during pregnancy: Three case reports. *Cancer Treat. Rep.*, **63**, 369–371

Donovan, P.B., Kaplan, M.E., Goldberg, J.D., Tatarsky, I., Najean, Y., Silberstein, E.B., Knospe, W.H., Laszlo, J., Mack, K., Berk, P.D. & Wasserman, L.R. (1984) Treatment of polycythemia vera with hydroxyurea. *Am. J. Hematol.*, **17**, 329–334

Editions du Vidal (1998) *Dictionnaire Vidal 1998*, 74th Ed., Paris, OVP, pp. 889–890

Epstein, S.S., Arnold, E., Andrea, J., Bass, W. & Bishop, Y. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol. appl. Pharmacol.*, **23**, 288–325

Evenson, D.P. & Jost, L.K. (1993) Hydroxyurea exposure alters mouse testicular kinetics and sperm chromatin structure. *Cell Prolif.*, **26**, 147–159

Fenech, M., Rinaldi, J. & Surralles, J. (1994) The origin of micronuclei induced by cytosine arabinoside and its synergistic interaction with hydroxyurea in human lymphocytes. *Mutagenesis*, **9**, 273–277

Ferguson, L.R. (1990) Mutagenic and recombinogenic consequences of DNA-repair inhibition during treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea in *Saccharomyces cerevisiae*. *Mutat. Res.*, **241**, 369–377

Ferguson, L.R. (1995) Mutagenic properties of anticancer drugs. In: Ponder, B.A.J. & Waring, M.J., eds, *The Genetics of Cancer*, Lancaster, Kluwer Academic Publisher, pp. 177–216

Ferguson, L.R. & Denny, W.A. (1995) Anticancer drugs: An underestimated risk or an underutilised resource in mutagenesis. *Mutat. Res.*, **331**, 1–26

Ferguson, L.R. & Turner, P.M. (1988a) 'Petite' mutagenesis by anticancer drugs in *Saccharomyces cerevisiae*. *Eur. J. Cancer clin. Oncol.*, **24**, 591–596

Ferguson, L.R. & Turner, P.M. (1988b) Mitotic crossing-over by anticancer drugs in *Saccharomyces cerevisiae* strain D5. *Mutat. Res.*, **204**, 239–249

Ferm, V.H. (1966) Severe developmental malformations. *Arch. Pathol.*, **81**, 174–177

Ferster, A., Vermylen, C., Cornu, G., Buyse, M., Corazza, F., Devalck, C., Fondu, P., Toppet, M. & Sariban, E. (1996) Hydroxyurea for treatment of severe sickle cell anemia: A pediatric clinical trial. *Blood*, **88**, 1960–1964

Fibach, E., Burke, K.P., Schechter, A.N., Noguchi, C.T. & Rodgers, G.P. (1993) Hydroxyurea increases fetal hemoglobin in cultured erythroid cells derived from normal individuals and patients with sickle cell anemia or beta-thalassemia. *Blood*, **81**, 1630–1635

Fitzgerald, J.M. & McCann, S.R. (1993) The combination of hydroxyurea and leucapheresis in the treatment of chronic myeloid leukaemia in pregnancy. *Clin. Lab. Haematol.*, **15**, 63–65

Foli, A., Maserati, R., Minoli, L., Wainberg, M.A., Gallo, R.C., Lisziewicz, J. & Lori, F. (1998) Therapeutic advantage of hydroxyurea and didanosine combination therapy in patients previously treated with zidovudine. *AIDS*, **12**, 1113–1114

Furgerson, J.L., Vukelja, S.J., Baker, W.J. & O'Rourke, T.J. (1996) Acute myeloid leukemia evolving from essential thrombocythemia in two patients treated with hydroxyurea. *Am. J. Hematol.*, **51**, 137–140

Galli, A. & Schiestl, R.H. (1996) Hydroxyurea induces recombination in dividing but not in G1 or G2 cell cycle arrested yeast cells. *Mutat. Res.*, **354**, 69–75

Gandhi, V., Plunkett, W., Kantarjian, H., Talpaz, M., Robertson, L.E. & O'Brien, S. (1998) Cellular pharmacodynamics and plasma pharmacokinetics of parenterally infused hydroxyurea during a phase I clinical trial in chronic myelogenous leukemia. *J. clin. Oncol.*, **16**, 2321–2331

Gao, W.Y., Zhou, B.S., Johns, D.G., Mitsuya, H. & Yen, Y. (1998) Role of the M2 subunit of ribonucleotide reductase in regulation by hydroxyurea of the activity of the anti-HIV-1 agent 2′,3′-dideoxyinosine. *Biochem. Pharmacol.*, **56**, 105–112

Gennaro, A.R. (1995) *Remington: The Science and Practice of Pharmacy*, 19th Ed., Easton, PA, Mack Publishing Co., Vol. II, p. 1252

Graham, P.J. (1955) *Synthesis of Ureas*. US Patent No. 2,705,727. Assigned to E.I. du Pont de Nemours & Co., Wilmington, DE

Greenwood, S.K., Armstrong, M.J., Hill, R.B., Bradt, C.I., Johnson, T.E., Hilliard, C.A. & Galloway, S.M. (1998) Fewer chromosome aberrations and earlier apoptosis induced by DNA synthesis inhibitors, a topoisomerase II inhibitor or alkylating agents in human cells with normal compared with mutant p53. *Mutat. Res*., **401**, 39–53

Gropper, C.A., Don, P.C. & Sadjadi, M.M. (1993) Nail and skin hyperpigmentation associated with hydroxyurea therapy for polycythemia vera. *Int. J. Dermatol.*, **32**, 731–733

Guilhot, F., Abgrall, J.-F., Harousseau, J.-L., Bauters, F., Brice, P., Dine, G., Tilly, H., Ifrah, N., Cassasus, P., Rochant, H., Christian, B., Guerci, A., Lamagnere, J.-P., Le Prise, P.-Y., Duclos, B. & Tanzer, J. (1993) A multicentric randomised study of alpha 2b interferon (IFN) and hydroxyurea (HU) with or without cytosine-arabinoside (Ara-c) in previously untreated patients with Ph+ chronic myelocytic leukemia (CML): Preliminary cytogenetic results. *Leuk. Lymphoma*, **11** (Suppl. 1), 181–183

Gwilt, P.R. & Tracewell, W.G. (1998) Pharmacokinetics and pharmacodynamics of hydroxyurea. *Clin. Pharmacokinet.*, **34**, 347–358

Hahn, P., Kapp, L.N., Morgan, W.F. & Painter, R.B. (1986) Chromosomal changes without DNA overproduction in hydroxyurea-treated mammalian cells: Implications for gene amplification. *Cancer Res*., **46**, 4607–4612

Hansen, D.K., Grafton, T.F., Cross, D.R. & James, S.J. (1995) Partial attenuation of hydroxyurea-induced embryotoxicity by deoxyribonucleotides in mouse and rat embryos treated *in vitro*. *Toxicol. in Vitro*, **9**, 11–19

Hart, J.W. & Hartley-Asp, B. (1983) Induction of micronuclei in the mouse. Revised timing of the final stage of erythropoiesis. *Mutat. Res*., **120**, 127–132

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutag*., **5** (Suppl. 1), 3–142

Herken, R. (1984) The influence of deoxycytidine monophosphate (dCMP) on the cytotoxicity of hydroxyurea in the embryonic spinal cord of the mouse. *Teratology*, **30**, 83–90

Herken, R. (1985) Ultrastructural changes in the neural tube of 10-day-old mouse embryos exposed to colchicine and hydroxyurea. *Teratology*, **31**, 345–352

Hill, A.B. & Schimke, R.T. (1985) Increased gene amplification in L5178Y mouse lymphoma cells with hydroxyurea-induced chromosomal aberrations. *Cancer Res*., **45**, 5050–5057

Holcombe, R.F., Treseler, P.A. & Rosenthal, D.S. (1991) Chronic myelomonocytic leukemia transformation in polycythemia vera. *Leukemia*, **5**, 606–610

Ishii, Y. & Bender, M.A. (1980) Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light-induced sister-chromatid exchanges in Chinese hamster cells. *Mutat. Res*., **79**, 19–32

Iversen, O.H. (1982a) Enhancement of methylnitrosourea skin carcinogenesis by inhibiting cell proliferation with hydroxyurea or skin extracts. *Carcinogenesis*, **3**, 881–889

Iversen, O.H. (1982b) Hydroxyurea enhances methylnitrosourea skin carcinogenesis when given shortly before, but not after, the carcinogen. *Carcinogenesis*, **3**, 891–894

Iwama, M., Sakamoto, Y., Honda, A. & Mori, Y. (1983) Limb deformity induced in chick embryo by hydroxyurea. *J. Pharmacobiodyn.*, **6**, 836–843

Jackson, H., Shukri, A. & Ali, K. (1993) Hydroxyurea treatment for chronic myeloid leukaemia during pregnancy. *Br. J. Haematol*., **85**, 203–204

Jackson, M.A., Stack, H.F. & Waters, M.D. (1996) Genetic activity profiles of anticancer drugs. *Mutat. Res.*, **355**, 171–208

Jelmert, Ø., Hansteen, I.-L. & Langård, S. (1992) Enhanced cytogenetic detection of previous in vivo exposure to mutagens in human lymphocytes after treatment with inhibitors of DNA synthesis and DNA repair *in vitro*. *Mutat. Res.*, **271**, 289–298

Jiang, J., Jordan, S.J., Barr, D.P., Gunther, M.R., Maeda, H. & Mason, R.P. (1997) In vivo production of nitric oxide in rats after administration of hydroxyurea. *Mol. Pharmacol.*, **52**, 1081–1086

Johns, D.G. & Gao, W.Y. (1998) Selective depletion of DNA precursors: An evolving strategy for potentiation of dideoxynucleoside activity against immunodeficiency virus. *Biochem. Pharmacol.*, **55**, 1551–1556

Kaplan, M.E., Mack, K., Goldberg, J.D., Donovan, P.B., Berk, P.D. & Wasserman, L.R. (1986) Long-term management of polycythemia vera with hydroxyurea: A progress report. *Semin. Hematol.*, **23**, 167–171

Karon, M. & Benedict, W.F. (1972) Chromatid breakage: Differential effect of inhibitors of DNA synthesis during G2 phase. *Science*, **178**, 62

Khera, K.S. (1979) A teratogenicity study on hydroxyurea and diphenylhydantoin in cats. *Teratology*, **20**, 447–452

Kihlman, B.A. & Andersson, H.C. (1985) Synergistic enhancement of the frequency of chromatid aberrations in cultured human lymphocytes by combinations of inhibitors of DNA repair. *Mutat. Res.*, **150**, 313–325

Kunze, E., Graewe, T., Scherber, S., Weber, J. & Gellhar, P. (1989) Cell cycle dependence of *N*-methyl-*N*-nitrosourea-induced tumour development in the proliferating, partially resected urinary bladder. *Br. J. exp. Pathol.*, **70**, 125–142

Lambert, B., Sten, M., Söderhäll, S., Ringborg, U. & Lewensohn, R. (1983) DNA repair replication, DNA breaks and sister-chromatid exchange in human cells treated with adriamycin *in vitro*. *Mutat. Res.*, **111**, 171–184

Landaw, S.A. (1986) Acute leukemia in polycythemia vera. *Semin. Hematol.*, **23**, 156–165

Levin, V.A. & Prados, M.D. (1992) Treatment of recurrent gliomas and metastatic brain tumors with a polydrug protocol designed to combat nitrosourea resistance. *J. clin. Oncol.*, **10**, 766–771

Li, J.C. & Kaminskas, E. (1987) Progressive formation of DNA lesions in cultured Ehrlich ascites tumor cells treated with hydroxyurea. *Cancer Res.*, **47**, 2755–2758

Liozon, E., Brigaudeau, C., Trimoreau, F., Desangles, F., Fermeaux, V., Praloran, V. & Bordessoule, D. (1997) Is treatment with hydroxyurea leukemogenic in patients with essential thrombocythemia? An analysis of three new cases of leukaemic transformation and review of the literature. *Hematol. Cell Ther.*, **39**, 11–18

Lisziewicz, J., Jessen, H., Finzi, D., Siliciano, R.F. & Lori, F. (1998) HIV-1 suppression by early treatment with hydroxyurea, didanosine, and a protease inhibitor. *Lancet*, **352**, 199–200

Löfvenberg, E. & Wahlin, A. (1988) Management of polycythaemia vera, essential thrombocythaemia and myelofibrosis with hydroxyurea. *Eur. J. Haematol.*, **41**, 375–381

Löfvenberg, E., Nordenson, I. & Wahlin, A. (1990) Cytogenetic abnormalities and leukemic transformation in hydroxyurea-treated patients with Philadelphia chromosome negative chronic myeloproliferative disease. *Cancer Genet. Cytogenet.*, **49**, 57–67

Lori, F., Gallo, R.C., Malykh, A., Cara, A., Romano, J., Markham, P. & Franchini, G. (1997a) Didanosine but not high doses of hydroxyurea rescue pigtail macaque from a lethal dose of SIV (smmpbj14). *AIDS Res. hum. Retroviruses*, **13**, 1083–1088

Lori, F., Malykh, A.G., Foli, A., Maserati, R., De Antoni, A., Minoli, L., Padrini, D., Degli Antoni, A., Barchi, E., Jessen, H., Wainberg, M.A., Gallo, R.C. & Lisziewicz, J. (1997b) Combination of a drug targeting the cell with a drug targeting the virus controls human immunodeficiency virus type 1 resistance. *AIDS Res. hum. Retroviruses*, **13**, 1403–1409

Loukopoulos, D., Voskaridou, E., Stamoulakatou, A., Papassotiriou, Y., Kalotychou, V., Loutradi, A., Cozma, G., Tsiarta, H. & Pavlides, N. (1998) Hydroxyurea therapy in thalassemia. *Ann. N.Y. Acad. Sci.*, **850**, 120–128

Luzzati, R., Di-Perri, G., Fendt, D., Ramarli, D., Broccali, G. & Concia, E. (1998) Pharmacokinetics, safety and anti-human immunodeficiency virus (HIV) activity of hydroxyurea in combination with didanosine (Letter). *J. antimicrob. Chemother.*, **42**, 565–566

Maier-Redelsperger, M., de Montalembert, M., Flahault, A., Neonato, M.G., Ducrocq, R., Masson, M.P., Girot, R. & Elion, J. for the French Study Group on Sickle Cell Disease (1998) Fetal hemoglobin and F-cell responses to long-term hydroxyurea treatment in young sickle cell patients. *Blood*, **91**, 4472–4479

Mattano, S.S., Palella, T.D. & Mitchell, B.S. (1990) Mutations induced at the hypoxanthine–guanine phosphoribosyltransferase locus of human T-lymphoblasts by perturbations of purine deoxyribonucleoside triphosphate pools. *Cancer Res.*, **50**, 4566–4571

Mayer, V.W., Goin, C.J. & Zimmermann, F.K. (1986) Aneuploidy and other genetic effects induced by hydroxyurea in *Saccharomyces cerevisiae*. *Mutat. Res.*, **160**, 19–26

Medical Economics Data Production (1999) *PDR®: Physicians' Desk Reference*, 53rd Ed., Montvale, NJ, Medical Economics

Mehnert, K., Vogel, W., Benz, R. & Speit, G. (1984) Different effects of mutagens on sister chromatid exchange induction in three Chinese hamster cell lines. *Environ. Mutag.*, **6**, 573–583

Millicovsky, G., DeSesso, J.M., Kleinman, L.I. & Clark, K.E. (1981) Effects of hydroxyurea on hemodynamics of pregnant rabbits: A maternally mediated mechanism of embryotoxicity. *Am. J. Obstet. Gynecol.*, **140**, 747–752

Minford, J., Kerrigan, D., Nichols, M., Shackney, S. & Zwelling, L.A. (1984) Enhancement of the DNA breakage and cytotoxic effects of intercalating agents by treatment with sublethal doses of 1-beta-D-arabinofuranosylcytosine or hydroxyurea in L1210 cells. *Cancer Res.*, **44**, 5583–5593

de Montalembert, M., Belloy, M., Bernaudin, F., Gouraud, F., Capdeville, R., Mardini, R., Philippe, N., Jais, J.P., Bardakdjian, J., Ducrocq, R., Maier-Redelsperger, M., Elion, J., Labie, D. & Girot, R. for the French Study Group on Sickle Cell Disease (1997) Three-year follow-up of hydroxyurea treatment in severely ill children with sickle cell disease. *J. pediatr. Hematol./Oncol.*, **19**, 313–318

Montaner, J.S., Zala, C., Conway, B., Raboud, J., Patenaude, P., Rae, S., O'Shaughnessy, M.V. & Schechter, M.T. (1997) A pilot study of hydroxyurea among patients with advanced human immunodeficiency virus (HIV) disease receiving chronic didanosine therapy: Canadian HIV trials network protocol 080. *J. infect. Dis.*, **175**, 801–806

Moschella, S.L. & Greenwald, M.A. (1973) Psoriasis with hydroxyurea. An 18-month study of 60 patients. *Arch. Dermatol.*, **107**, 363–368

Muranyi-Kovacs, I. & Rudali, G. (1972) Comparative study of carcinogenic activity of hydroxyurea and urethane in XVII-G mice. *Rev. Eur. Etud. clin. biol*., **17**, 93–95

Murphy, M.L. & Chaube, S. (1964) Preliminary survey of hydroxyurea (NSC-32065) as a teratogen. *Cancer Chemother. Rep.*, **40**, 1–7

Nair, R., Iyer, R.S., Nair, C.N., Kurkure, P.A., Pai, S.K., Saikia, T.K., Nadkarni, K.S., Pai, V.R., Gopal, R. & Advani, S.H. (1993) Myelodysplastic syndrome. A clinical and pathological analysis of 88 patients. *Indian J. Cancer*, **30**, 169–175

Najean, Y. & Rain, J.-D. (1997a) Treatment of polycythemia vera: The use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. *Blood*, **90**, 3370–3377

Najean, Y. & Rain, J.D. for the French Polycythemia Study Group (1997b) Treatment of polycythemia vera: Use of ^{32}P alone or in combination with maintenance therapy using hydroxyurea in 461 patients greater than 65 years of age. *Blood*, **89**, 2319–2327

Najean, Y., Rain, J.-D., Dresch, C., Goguel, A., Lejeune, F., Echard, M. & Grange, M.-J. (1996) Risk of leukaemia, carcinoma, and myelofibrosis in ^{32}P- or chemotherapy-treated patients with polycythemia vera: A prospective analysis of 682 cases. *Leuk. Lymphoma*, **22**, 111–119

Nand, S., Messmore, H., Fisher, S.G., Bird, M.L., Schulz, W. & Fisher, R.I. (1990) Leukemic transformation in polycythemia vera: Analysis of risk factors. *Am. J. Hematol*., **34**, 32–36

Nand, S., Stock, W., Godwin, J. & Fisher, S.G. (1996) Leukemogenic risk of hydroxyurea therapy in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Am. J. Hematol*., **52**, 42–46

Navarra, P., Del Carmine, R., Ciabattoni, G., D'Amato, M., Ragazzoni, E., Vacca, M., Volpe, A.R. & Preziosi, P. (1990) Hydroxyurea: Relationship between toxicity and centrally-induced adrenal activation. *Pharmacol. Toxicol.*, **67**, 209–215

Navarra, P., Puccetti, P., Riccardi, C. & Preziosi, P. (1995) Anticancer drug toxicity via cytokine production: The hydroxyurea paradigm. *Toxicol. Lett.*, **82–83**, 167–171

Navarra, P., Tringali, G. & Preziosi, P. (1996) The effects of inhibitors of cyclo-oxygenase, lipoxygenase and nitric oxide synthase pathways on the toxicity of hydroxyurea in adrenalectomized rats. *Toxicol. Lett.*, **86**, 13–18

Navarra, P., Grohmann, U., Nocentini, G., Tringali, G., Puccetti, P., Riccardi, C. & Preziosi, P. (1997) Hydroxyurea induces the gene expression and synthesis of proinflammatory cytokines *in vivo*. *J. Pharmacol. exp. Ther*., **280**, 477–482

Navarra, P., Tringali, G. & Preziosi, P. (1998) Hydroxyurea influences adrenocortical function in humans. *Eur. J. clin. Pharmacol.*, **54**, 491–492

Nyce, J., Liu, L. & Jones, P.A. (1986) Variable effects of DNA-synthesis inhibitors upon DNA methylation in mammalian cells. *Nucleic Acids Res*., **14**, 4353–4367

Ören, H., Düzovali, O., Yüksel, E., Sakizli, M. & Irken, G. (1999) Development of acute promyelocytic leukemia with isochromosome 17q after BCR/ABL positive chronic myeloid leukemia. *Cancer Genet. Cytogenet.*, **109**, 141–143

Painter, R.B. & Cleaver, J.E. (1967) Radiobiology. Repair replication in HeLa cells after large doses of X-irradiation. *Nature*, **216**, 369–370

Pajor, A., Zimonyi, I., Koos, R., Lehoczky, D. & Ambrus, C. (1991) Pregnancies and offspring in survivors of acute lymphoid leukemia and lymphoma. *Eur. J. Obstet. Gynecol. reprod. Biol*., **40**, 1–5

Palitti, F., Tanzarella, C., Degrassi, F., De Salvia, R., Fiore, M. & Natarajan, A.T. (1983) Formation of chromatid-type aberrations in G2 stage of the cell cycle. *Mutat. Res*., **110**, 343–350

Palitti, F., Tanzarella, C., Degrassi, F., De Salvia, R. & Fiore, M. (1984a) Enhancement of induced sister chromatid exchange and chromosomal aberrations by inhibitors of DNA repair processes. *Toxicol. Pathol*., **12**, 269–273

Palitti, F., Degrassi, F., De Salvia, R., Tanzarella, C. & Fiore, M. (1984b) Potentiation of induced sister chromatid exchanges and chromatid-type aberrations by inhibitors of DNA synthesis and repair in G2. *Basic Life Sci*., **29A**, 313–318

Patel, M., Dukes, I.A.F. & Hull, J.D. (1991) Use of hydroxyurea in chronic myeloid leukemia during pregnancy: A case report. *Am. J. Obstet. Gynecol*., **165**, 565–566

Philips, F.S. & Sternberg, S.S. (1975) Tests for tumour induction by antitumor agents. *Recent Results Cancer Res.*, **52**, 29–35

Popescu, N.C., Turnbull, D. & DiPaolo, J.A. (1977) Sister chromatid exchange and chromosome aberration analysis with the use of several carcinogens and noncarcinogens. *J. natl Cancer Inst*., **59**, 289–293

Pouchert, C.J. (1981) *The Aldrich Library of Infrared Spectra*, 3rd Ed., Milwaukee, WI, Aldrich Chemical Co., p. 475

Pouchert, C.J. (1983) *The Aldrich Library of NMR Spectra*, 2nd Ed., Milwaukee, WI, Aldrich Chemical Co., Vol. 1, p. 671

Pouchert, C.J. (1985) *The Aldrich Library of FT-IR Spectra*, Milwaukee, WI, Aldrich Chemical Co., Vol. 1, p. 801

Prempree, T. & Merz, T. (1969) Does hydroxyurea inhibit chromosome repair in cultured human lymphocytes? *Nature*, **224**, 603–604

Price, C.J., Tyl, R.W., Marks, T.A., Taschke, L.L., Ledoux, T.A. & Reel, J.R. (1985) Teratologic and postnatal evaluation of aniline hydrochloride in the Fischer 344 rat. *Toxicol. appl. Pharmacol*., **77**, 465–478

Quesnel, B., Kantarjian, H., Pedersen Bjergaard, J., Brault, P., Estey, E., Lai, J.L., Tilly, H., Stoppa, A.M., Archimbaud, E., Harousseau, J.L., Bauters, F. & Fenaux, P. (1993) Therapy-related acute myeloid leukemia with t(8;21), inv(16), and t(8;16): A report on 25 cases and review of the literature. *J. clin. Oncol.*, **11**, 2370–2379

Reiffers, J., Dachary, D., David, B., Bernard, P., Marit, G., Boisseau, M. & Brousted, A. (1985) Megakaryoblastic transformation of primary thrombocythemia. *Acta haematol.*, **73**, 228–231

Richard, M., Truchetet, F., Friedel, J., Leclech, C. & Heid, E. (1989) Skin lesions simulating chronic dermatomyositis during long-term hydroxyurea therapy. *J. Am. Acad. Dermatol.*, **21**, 797–799

Rodriguez, G.I., Kuhn, J.G., Weiss, G.R., Hilsenbeck, S.G., Eckardt, J.R., Thurman, A., Rinaldi, D.A., Hodges, S., Von Hoff, D.D. & Rowinsky, E.K. (1998) A bioavailability and pharmacokinetic study of oral and intravenous hydroxyurea. *Blood*, **91**, 1533–1541

Rossberger, S. & Andrae, U. (1985) DNA repair synthesis induced by *N*-hydroxyurea, acetohydroxamic acid, and *N*-hydroxyurethane in primary rat hepatocyte cultures: Comparative evaluation using the autoradiographic and the bromodeoxyuridine density-shift method. *Mutat. Res.*, **145**, 201–207

Rote Liste Sekretariat (1998) *Rote Liste 1998*, Frankfurt, Rote Liste Service GmbH, pp. 86-106, 86-115

Royal Pharmaceutical Society of Great Britain (1999) *Martindale, The Extra Pharmacopoeia*, 13th Ed., London, The Pharmaceutical Press [MicroMedex Online: Health Care Series]

Rutschmann, O.T., Opravil, M., Iten, A., Malinverni, R., Vernazza, P.L., Bucher, H.C., Bernasconi, E., Sudre, P., Leduc, D., Yerly, S., Perrin, L.H. & Hirschel, B. for the Swiss HIV Cohort Study (1998) A placebo-controlled trial of didanosine plus stavudine, with and without hydroxyurea, for HIV infection. *AIDS*, **12**, F71–F77

Sadtler Research Laboratories (1995) *Sadtler Standard Spectra, 1981–1995 Supplementary Index*, Philadelphia, PA, p. 1

Sato, K., Akaike, T., Sawa, T., Miyamoto, Y., Suga, M., Ando, M. & Maeda, H. (1997) Nitric oxide generation from hydroxyurea via copper-catalyzed peroxidation and implications for pharmacological actions of hydroxyurea. *Jpn. J. Cancer Res.*, **88**, 1199–1204

Schrell, U.M., Rittig, M.G., Anders, M., Koch, U.H., Marschalek, R., Kiesewetter, F. & Fahlbusch, R. (1997) Hydroxyurea for treatment of unresectable and recurrent meningiomas. II. Decrease in the size of meningiomas in patients treated with hydroxyurea. *J. Neurosurg.*, **86**, 840–844

Scott, W.J., Ritter, E.J. & Wilson, J.G. (1971) DNA synthesis inhibition and cell death associated with hydroxyurea teratogenesis in rat embryos. *Dev. Biol.*, **26**, 306–315

Scott, J.P., Hillery, C.A., Brown, E.R., Misiewicz, V. & Labotka, R.J. (1996) Hydroxyurea therapy in children severely affected with sickle cell disease. *J. Pediatr.*, **128**, 820–828

Sharon, R., Tatarsky, I. & Ben-Arieh, Y. (1986) Treatment of polycythemia vera with hydroxyurea. *Cancer*, **57**, 718–720

Simchen, G., Idar, D. & Kassir, Y. (1976) Recombination and hydroxyurea inhibition of DNA synthesis in yeast meiosis. *Mol. Gen. Genet.*, **144**, 21–27

Simonelli, C., Comar, M., Zanussi, S., De Paoli, P., Tirelli, U. & Giacca, M. (1997) No therapeutic advantage from didanosine (ddI) and hydroxyurea versus ddI alone in patients with HIV infection. *AIDS*, **11**, 1299–1300

Skog, S., Heiden, T., Eriksson, S., Wallström, B. & Tribukait, B. (1992) Hydroxyurea-induced cell death in human T lymphoma cells as related to imbalance in DNA/protein cycle and deoxyribonucleotide pools and DNA strand breaks. *Anticancer Drugs*, **3**, 379–386

Sofuni, T., Honma, M., Hayashi, M., Shimada, H., Tanaka, N., Wakuri, S., Awogi, T., Yamamoto, K.I., Nishi, Y. & Nakadate, M. (1996) Detection of in vitro clastogens and spindle poisons by the mouse lymphoma assay using the microwell method: Interim report of an international collaborative study. *Mutagenesis*, **11**, 349–355

Stasi, R., Cantonetti, M., Abruzzese, E., Papi, M., Didona, B., Cavalieri, R. & Papa, G. (1992) Multiple skin tumors in long-term treatment with hydroxyurea (Letter to the Editor). *Eur. J. Haematol.*, **48**,121–122

Stehman, F.B. (1992) Experience with hydroxyurea as a radiosensitizer in carcinoma of the cervix. *Semin. Oncol.*, **19**, 48–52

Stehman, F.B., Bundy, B.N., Kucera, P.R., Deppe, G., Reddy, S. & O'Connor, D.M. (1997) Hydroxyurea, 5-fluorouracil infusion, and cisplatin adjunct to radiation therapy in cervical carcinoma: A phase I–II trial of the Gynecologic Oncology Group. *Gynecol. Oncol.*, **66**, 262–267

Sterkers, Y., Preudhomme, C., Laï, J.-L., Demory, J.-L., Caulier, M.-T., Wattel, E., Borderssoule, D., Bauters, F. & Fenaux, P. (1998) Acute myeloid leukemia and myelodysplastic syndromes following essential thrombocythemia treated with hydroxyurea: High proportion of cases with 17p deletion. *Blood*, **91**, 616–622

Strauss, B., Coyle, M., McMahon, M., Kato, K. & Dolyniuk, M. (1972) DNA synthesis, repair and chromosome breaks in eucaryotic cells. *Johns Hopkins med. J.*, **1**, 111–124

Swiss Pharmaceutical Society, ed. (1999) *Index Nominum, International Drug Directory*, 16th Ed., Stuttgart, Medpharm Scientific Publishers [MicroMedex Online: Health Care Series]

Sylvester, R.K., Lobell, M., Teresi, M.E., Brundage, D. & Dubowy, R. (1987) Excretion of hydroxyurea into milk. *Cancer*, **60**, 2177–2178

Tanaka, M., Yamazaki, Y., Kondo, E., Hattori, M., Tsushita, K. & Utsumi, M. (1997) Achievement of a complete cytogenetic response with hydroxyurea in a patient with chronic myelogenous leukemia. *Leuk. Res.*, **21**, 465–468

Tefferi, A. (1998) Is hydroxyurea leukemagenic in essential thrombocythemia? (Letter to the Editor). *Blood*, **92**, 1459–1460; discussion 1460–1461

Tertian, G., Tchernia, G., Papiernik, E. & Elefant, E. (1992) Hydroxyurea and pregnancy (Letter to the Editor). *Am. J. Obstet. Gynecol.*, **166**, 1868

Theisen, C.T., Fradkin, R. & Wilson, J.G. (1973) Teratogenicity of hydroxyurea in rhesus monkeys. *Teratology*, **7**, A29

Thomas, J., ed. (1998) *Australian Prescription Products Guide*, 27th Ed., Victoria, Australian Pharmaceutical Publishing, Vol. 1, p. 1406

Timson, J. (1975) Hydroxyurea. *Mutat. Res.*, **32**, 115–132

Tohda, H. & Oikawa, A. (1990) Hypoxanthine enhances hydroxyurea-induced sister-chromatid exchanges in Chinese hamster ovary cells. *Mutat. Res.*, **230**, 235–240

Toraason, M., Bohrman, J.S., Krieg, E., Combes, R.D., Willington, S.E., Zajac, W. & Langenbach, R. (1992) Evaluation of the V79 cell metabolic co-operation assay as a screen in vitro for developmental toxicants. *Toxicol. in Vitro*, **6**, 165–174

Triadou, P., Maier-Redelsperger, M., Krishnamoorty, R., Deschamps, A., Casadevall, N., Dunda, O., Ducrocq, R., Elion, J., Girot, R., Labie, D., Dover, G. & Cornu, P. (1994) Fetal haemoglobin variations following hydroxyurea treatment in patients with cyanotic congenital heart disease. *Nouv. Rev. Fr. Hematol.*, **36**, 367–372

Tsutsui, T., Barrett, J.C. & Ts'o, P.O.P. (1979) Morphological transformation, DNA damage, and chromosomal aberrations induced by a direct DNA perturbation of synchronized Syrian hamster embryo cells. *Cancer Res.*, **39**, 2356–2365

US Pharmacopeial Convention (1994) *The 1995 US Pharmacopeia*, 23rd Rev./The National Formulary, 18th Rev., Rockville, MD, pp. 776–777

US Pharmacopeial Convention (1998) *USP Dispensing Information*, Vol. I, *Drug Information for the Health Care Professional*, 18th Ed., Rockville, MD, pp. 1607–1609

Van den Berg, C.L., McGill, J.R., Kuhn, J.G., Walsh, J.T., De La Cruz, P.S., Davidson, K.K., Wahl, G.M. & Von Hoff, D.D. (1994) Pharmacokinetics of hydroxyurea in nude mice. *Anticancer Drugs*, **5**, 573–578

Velez, A., Lopez-Rubio, F. & Moreno, J.C. (1998) Chronic hydroxyurea-induced dermatomyositis-like eruption with severe dermal elastosis. *Clin. exp. Dermatol.*, **23**, 94–95

Vila, J., Nugier, F., Bargues, G., Vallet, T., Peyramond, D., Hamedi-Sangsari, F. & Seigneurin, J.M. (1997) Absence of viral rebound after treatment of HIV-infected patients with didanosine and hydroxycarbamide. *Lancet*, **350**, 635–636

Villani, P., Maserati, R., Regazzi, M.B., Giacchino, R. & Lori, F. (1996) Pharmacokinetics of hydroxyurea in patients infected with human immunodeficiency virus type I. *J. clin. Pharmacol.*, **36**, 117–121

Vorhees, C.V., Butcher, R.E., Brunner, R.L. & Sobotka, T.J. (1979) A developmental test battery for neurobehavioral toxicity in rats: A preliminary analysis using monosodium glutamate calcium carrageenan, and hydroxyurea. *Toxicol. appl. Pharmacol.*, **50**, 267–282

Vorhees, C.V., Butcher, R.E., Brunner, R.L., Wootten, V. & Sobotka, T.J. (1983) Developmental toxicity of phychotoxicity of FD and C red dye No. 40 (allura red AC) in rats. *Toxicology*, **28**, 207–217

Voskaridou, E., Kalotychou, V. & Loukopoulos, D. (1995) Clinical and laboratory effects of long-term administration of hydroxyurea to patients with sickle-cell/beta-thalassaemia. *Br. J. Haematol.*, **89**, 479–484

Wadler, S., Haynes, H., Schechner, R., Rozenblit, A. & Wiernik, P.H. (1996) Phase I trial of high-dose infusional hydroxyurea, high-dose infusional 5-fluorouracil and recombinant interferon-alpha-2a in patients with advanced malignancies. *Invest. New Drugs*, **13**, 315–320

Wangenheim, J. & Bolcsfoldi, G. (1988) Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis*, **3**, 193–205

Warner, C.W., Sadler, T.W., Shockey, J. & Smith, M.K. (1983) A comparison of the in vivo and in vitro response of mammalian embryos to a teratogenic insult. *Toxicology*, **28**, 271–282

Weinfeld, A., Swolin, B. & Westin, J. (1994) Acute leukaemia after hydroxyurea therapy in polycythemia vera and allied disorders: Prospective study of efficacy and leukaemogenicity with therapeutic implications. *Eur. J. Haematol.*, **52**, 134–139

Weinlich, G., Schuler, G., Greil, R., Kofler, H. & Fritsch, P. (1998) Leg ulcers associated with long-term hydroxyurea therapy. *J. Am. Acad. Dermatol.*, **39**, 372–374

West, W.O. (1987) Hydroxyurea in the treatment of polycythemia vera: A prospective study of 100 patients over a 20-year period. *South. med. J.*, **80**, 323–327

Wiger, R., Hongslo, J.K., Evenson, D.P., De Angelis, P., Schwartze, P.E. & Holme, J.A. (1995) Effects of acetaminophen and hydroxyurea on spermatogenesis and sperm chromatin structure in laboratory mice. *Reprod. Toxicol.*, **9**, 21–33

Wilson, J.G. (1974) Teratogenic causation in man and its evaluation in non-human primates. In: Motulsky, A.G. & Lenz, W., eds, *Birth Defects. Proceedings of 4th International Conference*, Amsterdam, Excerpta Medica, pp. 191–203

Wilson, J.G., Scott, W.J., Ritter, E.J. & Fradkin, R. (1975) Comparative distribution and embryotoxicity of hydroxyurea in pregnant rats and rhesus monkeys. *Teratology*, **11**, 169–178

Zaborowska, D., Swietlinska, Z. & Zuk, J. (1983) Induction of mitotic recombination by UV and diepoxybutane and its enhancement by hydroxyurea in *Saccharomyces cerevisiae*. *Mutat. Res.*, **120**, 21–26