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A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

METHYL-CCNU

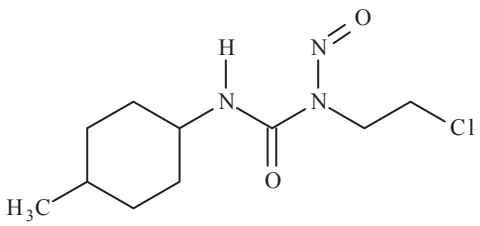
Methyl-CCNU was considered by previous IARC Working Groups in 1980 and 1987 (IARC, 1981, 1987). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 13909-09-6
Chem. Abstr. Name: Urea, *N*-(2-chloroethyl)-*N′*-(4-methylcyclohexyl)-*N*-nitroso-
IUPAC Systematic Name: 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
Synonyms: 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; 1-(2-chloroethyl)-3-(4-methylcyclohexyl) nitrosourea; methyl-CCNU; *N′*-(4-methylcyclohexyl)-*N*-(2-chloroethyl)-*N*-nitrosourea; semustine
Description: Light yellow powder (NTP, 2005)

1.1.1 Structural and molecular formulae, and relative molecular mass



$C_{10}H_{18}ClN_3O_2$
Relative molecular mass: 247.7

1.2 Use of the agent

1.2.1 Indications

Methyl-CCNU is an alkylating agent used alone or in combination with other chemotherapeutic agents to treat several types of cancers, including primary and metastatic brain tumours, Lewis lung tumour, and L1210 leukaemia. It has also been used to treat cancers of the digestive tract, Hodgkin lymphoma, malignant melanoma, and epidermoid carcinoma of the lung (NCI, 1979; US National Institutes of Health, 2000; NTP, 2005).

1.2.2 Dosage

Doses varied depending on the type of cancer and body weight of the individual. The typical oral dose was 125–200 mg/m^2 body surface area, and was repeated every 6 weeks (NTP, 2005). An alternative regimen was reported to be 200–225 mg/m^2 orally every 6–8 weeks (NCI, 1979).

Methyl-CCNU was available as 10, 50, and 100 mg capsules (NCI, 1979).

1.2.3 Trends in use

Methyl-CCNU was used in investigational studies in the 1960s and 1970s; it has never been approved as an antineoplastic drug, and is not listed in any of the standard pharmaceutical references and sources. However, some recently published articles indicate that it has been used in the People's Republic of China to treat various haematopoietic malignancies (Jia *et al.*, 2006; Zhang *et al.*, 2007; Guo *et al.*, 2008).

2. Cancer in Humans

The previous evaluation was based on five studies from the same set of patients described below. No new data were available to the Working Group.

Adjuvant treatment with methyl-CCNU was evaluated in 3633 patients with gastrointestinal cancer treated in nine randomized trials. Among 2067 patients treated with methyl-CCNU, 14 cases of acute myeloid leukaemia occurred, whereas one occurred among 1566 patients treated with other therapies (relative risk, 12.4; 95% confidence interval: 1.7–250). Cumulative (actuarial) risk was 4% at 6 years, and was not affected by concomitant radiotherapy or immunotherapy (Boice *et al.*, 1983). A subsequent report described a strong dose–response relationship, adjusted for survival time, giving a relative risk of almost 40 among patients who had received the highest dose (Boice *et al.*, 1986).

3. Cancer in Experimental Animals

Data on methyl-CCNU were included in a report in which a large number of cancer chemotherapeutic agents were tested for carcinogenicity by intraperitoneal injection in male Sprague-Dawley rats, and male Swiss mice. In rats injected with methyl-CCNU three times weekly for 6 months, the incidence of peritoneal sarcoma increased, and total tumour incidence was reported to increase 1.5-fold over that in controls at 18 months. No increase in tumour incidence was observed in mice (Weisburger, 1977). Intravenous administration of methyl-CCNU to rats was also reported to clearly induce lung tumours (Habs & Schmähl, 1984).

See Table 3.1

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In humans, following oral administration, methyl-CCNU is well absorbed from the gastrointestinal tract, undergoes rapid chemical decomposition and oxidative metabolism, and is rapidly distributed throughout the body (Sweetman, 2005). Following the oral administration of radiolabelled methyl-CCNU to cancer patients, radioactivity is detected in plasma within 10 minutes, with peak plasma levels attained within 3–6 hours. Radioactivity is slowly eliminated from the plasma, with a half-life of 36 hours reported for the chloroethyl moiety, and two half-lives for the cyclohexyl moiety: an initial half-life of 24 hours, and a subsequent half-life of 72 hours. Approximately 60% of the administered radioactivity is excreted in the urine within 48 hours (Sponzo *et al.*, 1973).

In addition to chemical degradation, methyl-CCNU is metabolized by the cytochrome P450 (CYP) mono-oxygenase system on the cyclohexyl ring carbons, and the 2-chloroethyl side-chain (Reed, 1994). CYP-dependent formation of alkylating metabolites from methyl-CCNU has been observed in studies *in vitro* with rat liver microsomes. The alkylating metabolites bind covalently to DNA and protein (Kramer, 1989; Reed, 1994), and increase mutagenicity in bacteria (Franza *et al.*, 1980). Methyl-CCNU is also metabolized to carbamylating species, but subsequent carbamoylation reactions are not

Table 3.1 Studies of cancer in experimental animals exposed to methyl-CCNU

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M) 18 mo Weisburger (1977)	i.p. 0, 1.5, 3.0 mg/kg 3 ×/wk for 6 mo 25/group 179 controls	Peritoneal sarcomas: 0/179 (0%), 3/49 (6.1%) Lung: 3/179 (1.1%), 3/49 (6.1%) Mammary gland: 4/179 (2.2%), 3/49 (6.1%) Brain: 2/179 (1.1%), 1/49 (2.0%)	[$P < 0.009$]	Purity NR Dosage groups were combined for each sex Overall increase in malignant tumours: 1.5-fold (53% vs 34%)
Rat, Wistar (M) Lifetime Habs & Schmähl (1984)	i.v. 0, 19, 38, 75, 150 mg/m^2 once every 6 wk 30/group 120 controls	Lung: +, [proven evidence according to the authors]		Purity > 99% (for clinical use)

i.p., intraperitoneal; i.v., intravenously; M, male; mo, month or months; NR, not reported; vs, versus; wk, week or weeks

significantly affected by enzymatic microsomal metabolism (Kramer, 1989; Reed, 1994).

4.2 Genotoxic effects

Methyl-CCNU is a bifunctional antineoplastic agent that undergoes spontaneous chemical decomposition yielding electrophilic compounds. These induce alkylation and carbamoylation of cellular macromolecules, including DNA and protein (Kramer *et al.*, 1986; Kramer, 1989; Reed, 1994). As with other chloroethylnitrosoureas, the majority of the alkylation reactions occur at the N^7 position of guanine, but the critical reaction leading to cytotoxicity is reported to involve alkylation of the O^6 of guanine, which leads to G–C cross-links in DNA (Chu & Sartorelli, 2007). Carbamoylation of proteins is also believed to contribute to the toxicity of methyl-CCNU, and may contribute to carcinogenesis through inhibition of DNA-repair processes (Kramer, 1989; Reed, 1994).

Methyl-CCNU has been tested for genotoxicity in several short-term assays *in vitro* and *in vivo*. The administration of methyl-CCNU *in vivo* results in DNA adducts in the bone marrow, spleen and colon of treated mice (Wheeler *et al.*, 1983), and in the kidney, liver and lung of treated rats (Kramer *et al.*, 1985, 1986). Large increases in the frequency of micronuclei are also seen in the bone-marrow erythrocytes of mice treated with methyl-CCNU (Tinwell & Ashby, 1991; Ashby *et al.*, 1993). Methyl-CCNU induces chromosomal aberrations, micronuclei, sister chromatid exchange, and DNA strand breaks in human or rodent cells *in vitro* (Erickson *et al.*, 1978; Wheeler *et al.*, 1983; Shah *et al.*, 1986; Baumler *et al.*, 1987; Vyas *et al.*, 1988; Tapiero *et al.*, 1989). It also induces mitotic crossing-over in yeast (Ferguson & Turner 1988a, b), and is mutagenic in bacteria (Auletta *et al.*, 1978; Franza *et al.*, 1980; Ashby *et al.*, 1993).

In addition, patients treated with methyl-CCNU (in combination with 5-fluorouracil and vincristine) as cytostatic therapy were found to have increased frequencies of sister chromatid exchange and chromosomal aberrations in their peripheral blood lymphocytes (Gebhart *et al.*, 1980a, b). Myelosuppression has also been reported in patients treated with methyl-CCNU (Young *et al.*, 1973; Breeden *et al.*, 1982).

4.3 Mechanisms of carcinogenesis

Acute myeloid leukaemia that develops in patients who have previously been treated with alkylating agents such as methyl-CCNU frequently exhibits distinctive characteristics that allow it to be distinguished from acute myeloid leukaemia induced by other agents (such as topoisomerase II inhibitors) or acute myeloid leukaemia that occurs spontaneously (Pedersen-Bjergaard & Rowley, 1994; Jaffe *et al.*, 2001; Smith *et al.*, 2003; Pedersen-Bjergaard *et al.*, 2006). One of the hallmarks of leukaemias induced by alkylating agents is that they frequently exhibit a clonal loss of either chromosome 5 or 7 (–5, –7) or a loss of part of the long arm of one of these chromosomes (5q–, 7q–). For example, a deletion within the long arm of chromosome 5 involving the bands q23 to q32 is often seen (Jaffe *et al.*, 2001).

In addition, mutations in *TP53* are frequently seen in leukaemias with the –5/5q– karyotype, and mutations involving the *AML1* gene as well as mutations in *TP53* and *RAS* are also seen in a subset of leukaemias that exhibit the –7/7q– karyotype (Christiansen *et al.*, 2001, 2005; Pedersen-Bjergaard *et al.*, 2006). These treatment-related acute myeloid leukaemias also frequently exhibit increased methylation of the *p15* promoter (Pedersen-Bjergaard *et al.*, 2006). Although methyl-CCNU has not been directly shown to induce losses or deletions affecting chromosomes 5 or 7, this drug has been reported to induce similar types of chromosomal alterations and deletions in a variety of experimental models (see description above), and in the lymphocytes of treated patients (Gebhart *et al.*, 1980a, b). The detection of elevated levels of chromosomal aberrations in the peripheral blood lymphocytes of patients treated with methyl-CCNU is of particular note, as multiple prospective studies have shown that individuals with increased levels of chromosomal aberrations in these cells are at increased risk of developing cancer (Hagmar *et al.*, 1998, 2004; Liou *et al.*, 1999; Smerhovsky *et al.*, 2001; Boffetta *et al.*, 2007).

4.4 Synthesis

Methyl-CCNU is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of methyl-CCNU. Methyl-CCNU causes acute myeloid leukaemia.

There is *limited evidence* in experimental animals for the carcinogenicity of methyl-CCNU.

Methyl-CCNU is *carcinogenic to humans (Group 1)*.

References

Ashby J, Vogel EW, Tinwell H *et al.* (1993). Mutagenicity to Salmonella, Drosophila and the mouse bone marrow of the human antineoplastic agent fotemustine: prediction of carcinogenic potency. *Mutat Res*, 286: 101–109. PMID:7678906

Auletta AE, Martz AG, Parmar AS (1978). Mutagenicity of nitrosourea compounds for Salmonella typhimurium. *J Natl Cancer Inst*, 60: 1495–1497. PMID:349171

Baumler T, Gebhart E, Tittelbach H (1987). Chromatid interchange, sister-chromatid exchange and micronuclei as indicators of clastogenic and anticlastogenic action in human lymphocyte cultures. *Mutat Res Envir Mutag Relat Subj*, 182: 275–276.

Boffetta P, van der Hel O, Norppa H *et al.* (2007). Chromosomal aberrations and cancer risk: results of a cohort study from Central Europe. *Am J Epidemiol*, 165: 36–43. doi:10.1093/aje/kwj367 PMID:17071846

Boice JD Jr, Greene MH, Killen JY Jr *et al.* (1983). Leukemia and preleukemia after adjuvant treatment of gastrointestinal cancer with semustine (methyl-CCNU). *N Engl J Med*, 309: 1079–1084. doi:10.1056/NEJM198311033091802 PMID:6353233

Boice JD, Greene MH, Killen JY Jr *et al.* (1986). Leukemia after adjuvant chemotherapy with semustine (methyl-CCNU)–evidence of a dose-response effect. *N Engl J*

Med, 314: 119–120. doi:10.1056/NEJM198601093140214 PMID:3941685

Breeden JH, Vollmer JT, Twomey PL (1982). Toxicity of very high dose nitrosourea administration. *Cancer*, 50: 1728–1733. doi:10.1002/1097-0142(19821101)50:9<1728::AID-CNCR2820500913>3.0.CO;2-N PMID:7116300

Christiansen DH, Andersen MK, Desta F, Pedersen-Bjergaard J (2005). Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia*, 19: 2232–2240. doi:10.1038/sj.leu.2404009 PMID:16281072

Christiansen DH, Andersen MK, Pedersen-Bjergaard J (2001). Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. *J Clin Oncol*, 19: 1405–1413. PMID:11230485

Chu E, Sartorelli AC (2007). *Cancer chemotherapy*. In: *Basic and Clinical Pharmacology*, 10th ed. Katzung BG, editor. New York: McGraw Hill Medical.

Erickson LC, Osieka R, Kohn KW (1978). Differential repair of 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea-induced DNA damage in two human colon tumor cell lines. *Cancer Res*, 38: 802–808. PMID:626984

Ferguson LR & Turner PM (1988a). Mitotic crossing-over by anticancer drugs in Saccharomyces cerevisiae strain D5. *Mutat Res*, 204: 239–249. doi:10.1016/0165-1218(88)90095-X PMID:3278216

Ferguson LR & Turner PM (1988b). 'Petite' mutagenesis by anticancer drugs in Saccharomyces cerevisiae. *Eur J Cancer Clin Oncol*, 24: 591–596. doi:10.1016/0277-5379(88)90286-6 PMID:3289944

Franza BR Jr, Oeschger NS, Oeschger MP, Schein PS (1980). Mutagenic activity of nitrosourea antitumor agents. *J Natl Cancer Inst*, 65: 149–154. PMID:6446611

Gebhart E, Lösing J, Wopfner F (1980a). Chromosome studies on lymphocytes of patients under cytostatic therapy. I. Conventional chromosome studies in cytostatic interval therapy. *Hum Genet*, 55: 53–63. doi:10.1007/BF00329127 PMID:7450757

Gebhart E, Windolph B, Wopfner F (1980b). Chromosome studies on lymphocytes of patients under cytostatic therapy. II. Studies Using the BUDR-labelling technique in cytostatic interval therapy. *Hum Genet*, 56: 157–167. doi:10.1007/BF00295688 PMID:7450772

Guo Y, Lu JJ, Ma X *et al.* (2008). Combined chemoradiation for the management of nasal natural killer (NK)/T-cell lymphoma: elucidating the significance of systemic chemotherapy. *Oral Oncol*, 44: 23–30. doi:10.1016/j.oraloncology.2006.11.020 PMID:17306611

Habs M & Schmähl D (1984). Long-term toxic and carcinogenic effects of cytostatic drugs. *Dev Oncol*, 15: 201–209.

Hagmar L, Bonassi S, Strömberg U *et al.* (1998). Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res*, 58: 4117–4121. PMID:9751622

Hagmar L, Strömberg U, Bonassi S *et al.* (2004). Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. *Cancer Res*, 64: 2258–2263. doi:10.1158/0008-5472.CAN-03-3360 PMID:15026371

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203

IARC (1981). Some antineoplastic and immunosuppressive agents. *IARC Monogr Eval Carcinog Risk Chem Hum*, 26: 1–411. PMID:6944253

Jaffe ES, Harris NL, Stein H, Vardiman JW, editors (2001). *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues: WHO Classification of Tumours*. Lyon, France: IARC Press, pp. 89–91.

Jia YQ, Liu T, Xu CG *et al.* (2006). Comparison of the long term results between two conditioning regimens MCC and BuCy in chronic myelocytic leukemia after allogeneic stem cell transplantation. *Sichuan Da Xue Xue Bao Yi Xue Ban*, 37: 226–229. PMID:16608081

Kramer RA (1989). Cytochrome P-450-dependent formation of alkylating metabolites of the 2-chloroethylnitrosoureas MeCCNU and CCNU. *Biochem Pharmacol*, 38: 3185–3192. doi:10.1016/0006-2952(89)90612-6 PMID:2818619

Kramer RA, McMenamin MG, Boyd MR (1985). Differential distribution and covalent binding of two labeled forms of methyl-CCNU in the Fischer 344 rat. *Cancer Chemother Pharmacol*, 14: 150–155. doi:10.1007/BF00434355 PMID:3971479

Kramer RA, McMenamin MG, Boyd MR (1986). In vivo studies on the relationship between hepatic metabolism and the renal toxicity of 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (MeCCNU). *Toxicol Appl Pharmacol*, 85: 221–230. doi:10.1016/0041-008X(86)90116-X PMID:3764909

Liou S-H, Lung J-C, Chen Y-H *et al.* (1999). Increased chromosome-type chromosome aberration frequencies as biomarkers of cancer risk in a blackfoot endemic area. *Cancer Res*, 59: 1481–1484. PMID:10197617

NCI (1979). *Guidelines for the Clinical Use of Methyl CCNU (Semustine) (NSC 95441)*, Protocol #181–2. Bethesda, MD: Investigational Drug Branch/Cancer Therapy Evaluation Program/Division of Cancer Treatment, 9 pp.

NTP (2005). *11th Report on Carcinogens*, Research Triangle Park, NC.

Pedersen-Bjergaard J, Christiansen DH, Desta F, Andersen MK (2006). Alternative genetic pathways and cooperating genetic abnormalities in the pathogenesis of

therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia*, 20: 1943–1949. doi:10.1038/sj.leu.2404381 PMID:16990778

Pedersen-Bjergaard J & Rowley JD (1994). The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation. *Blood*, 83: 2780–2786. PMID:8180374

Reed DJ (1994). *The metabolism and mode of action of 2-chloroethylnitrosoureas*. In: *Anticancer Drugs: Reactive Metabolism and Drug Interactions*. Powis G, editor. Pergamon Press, 187–207.

Shah VC, Vyas RC, Adhvaryu SG (1986). Cytogenetic effects of Me-CCNU on sister chromatid exchange frequency, cellular kinetics and chromosome aberrations. *Neoplasma*, 33: 33–37. PMID:3754313

Smerhovsky Z, Landa K, Rössner P *et al.* (2001). Risk of cancer in an occupationally exposed cohort with increased level of chromosomal aberrations. *Environ Health Perspect*, 109: 41–45. doi:10.2307/3434919 PMID:11171523

Smith SM, Le Beau MM, Huo D *et al.* (2003). Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood*, 102: 43–52. doi:10.1182/blood-2002-11-3343 PMID:12623843

Sponzo RW, DeVita VT, Oliverio VT (1973). Physiologic disposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea (Me CCNU) in man. *Cancer*, 31:1154–1159.doi:10.1002/1097-0142(197305)31:5<1154::AID-CNCR2820310517>3.0.CO;2-B PMID:4705152

Sweetman SC, editor (2005). *Martindale: the Complete Drug Reference,* 34th ed. London: Pharmaceutical Press.

Tapiero H, Yin MB, Catalin J *et al.* (1989). Cytotoxicity and DNA damaging effects of a new nitrosourea, fotemustine, diethyl- 1-(3-(2-chloroethyl)-3-nitrosoureido) ethylphosphonate-S10036. *Anticancer Res*, 9: 1617–1622. PMID:2627116

Tinwell H & Ashby J (1991). Activity of the human carcinogen MeCCNU in the mouse bone marrow micronucleus assay. *Environ Mol Mutagen*, 17: 152–154. doi:10.1002/em.2850170303 PMID:2022193

US National Institutes of Health (2000). *Safety Data Sheet: Methyl-CCNU (Semustine).* Bethesda, MD.

Vyas RC, Adhvaryu SG, Shah VC (1988). Genotoxic effects of MeCCNU on human peripheral blood lymphocytes. *Toxicol Lett*, 44: 153–159. doi:10.1016/0378-4274(88)90141-5 PMID:3188073

Weisburger EK (1977). Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. *Cancer*, 40: Suppl1935–1949. doi:10.1002/1097-0142(197710)40:4+<1935::AID-CNCR2820400827>3.0.CO;2-R PMID:907995

Wheeler GP, Bowdon BJ, Torbert JW *et al.* (1983). Biological and biochemical effects of N-(2-chloroethyl)-N'-(trans-4-methylcyclohexyl)-N-nitrosourea on two transplantable murine colon tumors. *Cancer Res*, 43: 5837–5845. PMID:6640534

Young RC, Walker MD, Canellos GP *et al.* (1973). Initial clinical trials with methyl-CCNU 1-(2-chlorethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea (MeCCNU). *Cancer*, 31:1164–1169.doi:10.1002/1097-0142(197305)31:5<1164::AID-CNCR2820310519>3.0.CO;2-4 PMID:4705154

Zhang XH, Huang XJ, Liu KY *et al.* (2007). Modified conditioning regimen busulfan-cyclophosphamide followed by allogeneic stem cell transplantation in patients with multiple myeloma. *Chin Med J (Engl)*, 120: 463–468. PMID:17439737