NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for AZACITIDINE

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NTP Report on Carcinogens Listing for Azacitidine

Carcinogenicity

Azacitidine (5-Azacytidine; 5-AzaC) is *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals (NCI 42, 1978; Luz and Murray, 1988; IARC, 1990).

5-AzaC, when administered by intraperitoneal (i.p.) injection, induced lymphoreticular neoplasms and skin and lung tumors in male and/or female mice (NCI 42, 1978; Luz and Murray, 1988; multiple studies reviewed in IARC, 1990), and leukemia, lymphoma, and tumors of the liver and lung in offspring of treated pregnant dams (IARC, 1990). In male rats, 5-AzaC administered i.p. induced squamous cell carcinoma of the skin and interstitial-cell tumors of the testes, and appeared to increase the incidence of non-testis tumors in male offspring of treated dams (IARC, 1990).

There are no adequate data available to evaluate the carcinogenicity of 5-AzaC in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Using an initiation-promotion experimental design, a chronic i.p. treatment of male rats acutely administered *N*-nitrosodiethylamine (diethylnitrosamine; DEN) after partial hepatectomy with 5-AzaC synergistically increased the frequency of liver tumors and of lung and skin tumors (Carr et al., 1988; IARC, 1990).

The carcinogenic/enhancement activity of 5-AzaC has been postulated to result directly or indirectly from its ability to inhibit DNA methylation (Harrison et al., 1983; for reviews, see Kerbel et al., 1984; Kerbel et al., 1986; Takenaga, 1986; Glover et al., 1987; Glover and Leyland-Jones, 1987; Jones and Buckley, 1990; Haaf, 1995). Altered levels of DNA methylation can affect gene expression (for reviews see Cedar, 1988; IARC, 1990; Fajkus et al., 1992; Velge et al., 1995), with hypomethylation being associated with the expression of genes that are normally silent or downregulated (Jones et al., 1983; Nyce et al., 1983; Riggs and Jones, 1983; Collard et al., 1989; Jones and Buckley, 1990; Pascale et al., 1993). If any of these overexpressed genes are growth regulators, their overexpression would likely increase tumorigenic potential. Consistent with this hypothesis, treatment of noninvasive BW5147 T-lymphoma cells *in vitro* with 5-AzaC resulted in invasive and metastatic cells that exhibited persistent genomic hypomethylation (Collard et al., 1989; Habets et al., 1990). In addition, 5-AzaC in the absence of metabolic activation is positive in a wide variety of prokaryotic, lower eukaryotic, and mammalian *in vitro* test systems, inducing DNA damage, mutations (base-pair substitution mutations only) in prokaryote systems; mitotic recombination, gene conversion, and gene mutations in somatic and germ cells of lower eukaryotes (yeast, *Drosophila*, plants); and DNA damage, chromosomal aberrations, mutations (but not point), and morphological transformation in cultured mammalian cells. Studies to evaluate the genetic activity of 5-AzaC in somatic cells of mammals have not been reported; however, it was reported as negative for dominant lethal mutations in mice.

There are no data available to suggest that the mechanisms thought to account for tumor induction by 5-AzaC in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded; or

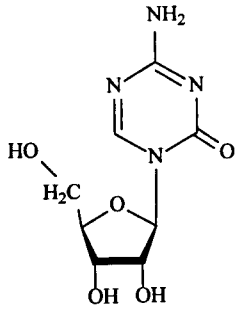
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

Azacitidine
[320-67-2]



1.1 Chemical Identification

Azacitidine ($C_8H_{12}N_4O_5$, mol. wt. = 224.2), which will be referred to as 5-AzaC in this report, is also called:

1,3,5-Triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl- (9CI)
s-Triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl- (8CI)
5-AC
4-Amino-1-β-D-ribofuranosyl-1,3,5-triazine-2(1*H*)-one
4-Amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one
4-Amino-1-β-D-ribofuranosyl-D-triazin-2(1*H*)-one
4-Amino-1-β-D-ribofuranosyl-*s*-triazin-2(1*H*)-one
Antibiotic U 18496
5-Azacytidine
5'-Azacytidin
5 Azc
5-Azcr
Ladakamycin
Mylosar
NCI-C01569
NSC 102816

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	White	Winkley and Robins(1970; cited by IARC, 1990)
Physical State	Crystals	Budavari (1996)
Melting Point, °C	228-230	Budavari (1996)
Solubility:		
Water at 20 °C	Soluble in warm water (40 mg/mL), cold water (14 mg/mL), 0.1 N hydrochloric acid (28 mg/mL), and 0.1 N sodium hydroxide (43 mg/mL)	von Hoff et al. (1975; cited by IARC, 1990)
Organic Solvents	Soluble in 35% ethanol (14.2-15.0 mg/mL), acetone (1 mg/mL), chloroform (1 mg/mL), hexane (1 mg/mL), and dimethyl sulfoxide (52.7 mg/mL)	von Hoff et al. (1975; cited by IARC, 1990)

1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

5-Azacytidine triphosphate
5-Azacuracil
5-Azauridine
Biuret
1-Formylbiuret
1-β-Ribofuranosyl-3-guanylurea (Guanylurea ribonucleoside)
α-D-Ribofuro(1',2':4,5)-2-azolidon
D-Ribose
Urea

Biuret is soluble in water, freely soluble in alcohol, and very slightly soluble in ether. D-Ribose is soluble in water and slightly soluble in alcohol. Urea is soluble in water, alcohol, methanol, glycerol, and concentrated HCl. It is insoluble in chloroform and ether (Budavari, 1996).

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Data, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 HUMAN EXPOSURE

2.1 Use

5-AzaC is a cytostatic agent that has been mainly used as an investigational drug since the 1970s for the treatment of acute leukemia. It is administered via intravenous (i.v.) and intramuscular (i.m.) injection and i.v. infusion, at a daily level of 40 to 750 mg/m^2. It is used

alone or in combination with vincristine, vinblastine, prednisone, cytarabine, or amsacrine, at a daily dose of 50-150 mg 5-AzaC/m^2. 5-AzaC has also been tested for use in the treatment of a variety of solid tumors (IARC, 1990).

5-AzaC has been used clinically in cancer treatment trial protocols in combinations with other antineoplastic agents such as doxorubicin, amasacrine and etoposide, and Granulocyte Colony Stimulating Factor (NCI/PDQ, 1996).

2.1 Production

5-AzaC is synthesized in Germany (IARC, 1990). 5-AzaC can be prepared by synthetic methods or can be isolated from a culture of the bacterium *Streptoverticillium ladakanus* (IARC, 1990). No data on imports or exports of 5-AzaC were available. The 1993 Chem Sources USA directory identified 13 U.S. suppliers and 2 foreign suppliers of 5-AzaC (Chem Sources USA, 1993). Chem Sources (1996) listed 14 U.S. suppliers, including the NCI Chemical Carcinogens Reference Standard Repository.

2.3 Environmental Exposure

Potential occupational exposure may occur for workers formulating or packaging 5-AzaC solutions and for health care professionals administering the drug. The National Occupational Exposure Survey (1981-1983) indicated that 1,069 workers, including 699 women, were potentially exposed to 5-AzaC (NIOSH, 1984). This estimate was derived from observations of the use of the actual compound (48% of total observations) and tradename products (52%).

2.4 Regulations

According to a monograph in the 1996 *Handbook on Injectable Drugs (HID)* entitled "Azacitidine Investigational" (Drug Information Fulltext, 1996), 5-AzaC is still an investigational drug. Its investigational number is NSC-102816. No regulatory information was found in the 1996 *Code of Federal Regulations* titles 21, 29, or 40.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of 5-AzaC in humans.

4.0 MAMMALIAN CARCINOGENICITY

Experimental details for the studies described in this section are presented in Table 4-1.

Summary: Treatment with 5-AzaC by intraperitoneal (i.p.) injection induced lung tumors in male and female A/He mice treated three times per week for 8 weeks (Stoner et al., 1973; cited by IARC, 1990), and leukemia in female AKR mice treated for 50 days (Vesely and Cihák, 1973; cited by IARC, 1990). In female BALB/c/A Bom Nhg (BALB/c) and CBA/Ca HSU Nhg (CBA) mice administered 5-AzaC i.p. for 21 weeks, an "unusual lymphoblastic leukemia-like lesion" was observed in the CBA group at 161 days (Luz and Murray, 1988). The incidences of lymphoreticular neoplasms and skin tumors were significantly increased in male and female BALB/c/Cb/Se mice administered 5-AzaC for 50 weeks (Cavaliere et al., 1987; cited by IARC, 1990). In males, but not females, the incidence of pulmonary adenoma was also significantly increased and in females, the incidence of adenocarcinoma and "adenocanthoma" of the mammary gland was significantly increased. The incidence of lymphocytic and granulocytic

neoplasms of the hematopoietic system was significantly increased in female, but not male, B6C3F1 mice administered 5-AzaC for 52 weeks (NCI 42, 1978; IARC, 1990). When offspring of pregnant dams treated with 5-AzaC i.p. on days 12, 14, or 16 of gestation were examined after 1 year, increased combined incidences of leukemia and lymphoma, liver tumors (unspecified types), and lung tumors (unspecified types) were present (Schmahl et al., 1985; cited by IARC, 1990).

In male F344 rats (females not evaluated) administered 5-AzaC i.p. for 9 months, interstitial-cell tumors of the testes and squamous cell carcinoma of the skin were detected in some 5-AzaC-treated rats but in none of the controls, but no mention was made of statistical analysis (Carr et al., 1984; IARC, 1990). The incidence of interstitial-cell tumors of the testes was significantly increased in male rats administered 5-AzaC i.p. for 1 year (Carr et al., 1988; IARC, 1990). Testes in male offspring whose dams were administered 5-AzaC i.p. on day 21 of gestation did not show an increased incidence of tumors 1 year after birth; however, the incidence of non-testis tumors (1 lung, 1 skin, 1 lymphoma) was significantly increased at 1 year (Carr et al., 1988).

4.1 Mice

4.1.1 Intraperitoneal Injection

In a screening assay that used A/He mice (which are highly susceptible to lung tumors), the number of macroscopic lung tumors was significantly increased in males and females administered 5-AzaC (3.7 mg/kg bw [15 μmol/kg bw]) i.p. 3 times per week for 8 weeks, beginning at age 6 to 8 weeks. The number of lung tumors was not increased significantly, however, in mice administered lower doses of 5-AzaC (1.4 or 2.6 mg/kg bw [5.7 or 11 μmol/kg bw]) in the same manner. Lung tumors (type not specified) were detected in 8/19 (42%) low-dose, 5/15 (33%) mid-dose, and 6/11 (54%) high-dose mice ([not broken down by sex] vs. 22% of untreated male controls, 17% of untreated female controls, 26% of male vehicle controls, and 23% of female vehicle controls). Actual tumor incidence in controls was not given in the original paper; no mention was made of the statistical analysis methodology in IARC (Stoner et al., 1973; cited by IARC, 1990).

In another screening assay, female AKR mice (highly susceptible to leukemia) were administered 5-AzaC (1.5 mg/kg bw [6.1 μmol/kg bw] 6 times over 20 days and then 0.8 mg/kg bw [3.3 μmol/kg bw] 6 times over the following 30 days) i.p., beginning at age 8 weeks. All 5-AzaC-treated mice died of leukemia by 60 days. Leukemia was not detected, however, in any of the controls during the observation period of 120 days and no control deaths were reported during this period. It was not specified if controls were injected with vehicle or were left untreated (Vesely and Cihák, 1973; cited by IARC, 1990).

In a study conducted by Luz and Murray (1988), female BALB/c/A Bom Nhg (BALB/c) and CBA/Ca HSU Nhg (CBA) mice were administered 5-AzaC (1 mg/kg [4 μmol/kg bw] i.p.; 11 injections over a 21-week period ["injections at weekly intervals until week 4, at 3 weekly intervals after week 6"]). At 161 days after start of the injection series (14 days after the eleventh injection of 5-AzaC), the first case of an unusual lymphoblastic leukemia-like lesion was observed in the CBA group. On days 163 and 164, the rate of occurrence increased suddenly, with 29 [of 50] mice dying or becoming moribund. Of the 5-AzaC-treated CBA mice that survived the treatment period, 34/47 (vs. 0/50 CBA controls) developed the lesion, the last case

occurring on day 189. None of the 5-AzaC-treated BALB/c mice or BALB/c controls developed the lesion. Statistical analysis of tumor incidence was not performed and the age of the mice at treatment initiation was not specified.

The incidences of lymphoreticular neoplasms and skin tumors (types not specified) were significantly increased in male and female BALB/c/Cb/Se mice administered 5-AzaC (2.0 mg/kg bw [8.2 μmol/kg bw]) i.p. once per week for 50 weeks, beginning at age 8 weeks. In males, but not females, the incidence of pulmonary adenoma was also significantly increased and in females, the incidence of adenocarcinoma and "adenocanthoma" of the mammary gland was significantly increased (the term adenocanthoma is not normally used to describe mammary tumors) (Cavaliere et al., 1987; cited by IARC, 1990).

The incidence of lymphocytic and granulocytic neoplasms of the hematopoietic system (sarcoma, leukemia, malignant lymphoma) was significantly increased in female, but not male, B6C3F1 mice administered 5-AzaC (2.2 mg/kg bw [9.0 μmol/kg bw]) i.p. 3 times per week for 52 weeks, beginning at age 38 days. When a higher dose (4.4 mg/kg bw [18 μmol/kg bw]) was administered in the same manner, there was no significant increase in the incidence of lymphocytic and granulocytic neoplasms of the hematopoietic system in males or females, although early deaths in the high-dose group may have precluded the development of neoplasia. One female in the high-dose group developed a basal-cell carcinoma (NCI 42, 1978; IARC, 1990).

4.1.2 Transplacental Administration

In a study conducted by Schmahl et al. (1985; cited by IARC, 1990), 5 pregnant NMRI mice were administered 5-AzaC (1 or 2 mg/kg bw [4 or 8 μmol/kg bw]) i.p. on day 12, 14, or 16 of gestation. The combined incidence of leukemia and lymphoma was increased only in 1-mg offspring treated with 5-AzaC beginning on day 12 or day 16 of gestation and in 2-mg offspring treated with 5-AzaC beginning on day 16 of gestation. The incidence of lung tumors (type not specified) was increased only in 1-mg offspring treated with 5-AzaC beginning on day 16 of gestation and in 2-mg offspring treated with 5-AzaC beginning on day 14 or 16 of gestation. The incidence of liver tumors (type not specified) was increased in all groups except 1-mg female offspring of dams treated with 5-AzaC on day 12 and 2-mg female offspring of dams treated with 5-AzaC on day 16 (see Table 4-1 for details). It was also reported in the IARC review that "some increases in the incidence of soft-tissue sarcomas were also seen," but no details were given.

4.2 Rats

4.2.1 Intraperitoneal Injection

In the rodent bioassay conducted by the National Cancer Institute (NCI 42, 1978), Sprague-Dawley rats (35/sex/dose) were given 5-AzaC in i.p. doses of 2.6 or 5.2 mg/kg bw (11 or 21 μmol/kg bw) three times per week for 34 weeks followed by an observation period of 46 or 47 weeks. Surviving rats were sacrificed at 80 or 81 weeks. Vehicle controls (15 per sex) received buffered saline. A group of untreated controls also comprised 15 rats of each sex. Tumors were observed in one high-dose male and three high-dose female rats. The incidence of tumors in the low-dose rats was not significantly increased as calculated by statistical test. The

short life span and short treatment periods due to high mortality in the high-dose groups precluded evaluation of the carcinogenicity of 5-AzaC in male and female rats.

In a study that seemed to have no controls, it was reported that the incidences of testes tumors and non-testis tumors were "not significantly" increased in male F344 rats administered a single i.p. dose of 5-AzaC (5 mg/kg bw [20.5 μmol/kg bw]) at age 21 days and examined at age 1 year (Carr et al., 1988).

In 160- to 180-g male F344 rats (age not specified) administered 5-AzaC (2.5 or 10 mg/kg bw [10 or 41 μmol/kg bw]) i.p. twice per week for 9 months, interstitial-cell tumors of the testes were detected in 9/12 low-dose and 1/8 high-dose rats (vs. 0/12 controls), squamous cell carcinomas were detected in 2/8 high-dose rats, but in no other groups, and a "skin appendage tumor at the site of injection" was detected in 1/8 high-dose rats, but in no other groups. No mention was made of statistical analysis (Carr et al., 1984; cited by IARC, 1990).

The incidence of interstitial-cell tumors of the testes was significantly increased in 160-180 g male F344 rats (age not specified) administered 5-AzaC (2.5 mg/kg bw [10 μmol/kg bw]) i.p. 3 times per week for 1 year. Other tumors detected were: 4 lymphomas, 4 renal tumors, 1 lung tumor, 3 skin tumors, 2 mesotheliomas, and 2 sarcomas. No other details about these tumors were given. In rats that were administered lower doses of 5-AzaC (0.025 or 0.25 mg/kg bw [0.1 or 1.0 μmol/kg bw]) in the same manner, the incidence of testes tumors was not significantly increased and no non-testis tumors were detected (Carr et al., 1988; IARC, 1990).

4.2.2 Transplacental Administration

In a study conducted by Carr et al. (1988), pregnant F344 rats were administered a single i.p. dose of 5-AzaC (10 mg/kg bw [41 μmol/kg bw]) on day 21 of pregnancy. It was reported that the incidence of non-testis tumors was increased in 1-year-old male and female offspring (3/22 vs. 0/49 in the controls) and that the incidence of tumors of the testes in 1-year-old male offspring was not significantly increased. There was no mention, however, of controls.

Table 4-1. Mammalian Carcinogenicity of Azacitidine

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - *Intraperitoneal Injection*							
6- to 8-wk old A/He mice	10M, 10F per dose	10M, 10F (vehicle alone) 10M, 10F (untreated)	5-AzaC, purity not specified	"total dose": 33, 62, or 90 mg/kg bw* (135, 254, or 368 µmol/kg bw), administered i.p. 3 times/wk over 8 wk * maximum tolerated dose The vehicle was composed of saline, polysorbate-80, carboxymethyl cellulose, and benzyl alcohol.	8 wk	This study was a screening assay. A/He mice are highly susceptible to lung tumors. All mice were killed 24 weeks after the first injection. Lung tumor incidence within each dose was combined for males and females and was calculated based on the number of survivors. Statistical analysis of tumor incidence was not mentioned in the IARC review. **Lungs:** Lung tumors (type not specified) were detected in 8/19 (42%) LD, 5/15 (33%) MD, and 6/11 (54%) HD mice (vs. 22% of untreated male controls, 17% of untreated female controls, 26% of male vehicle controls, and 23% of female vehicle controls). Actual tumor incidence in controls was not given. The tumor multiplicity in mice of each sex treated with the highest dose was significantly increased ($p < 0.05$) (0.73 ± 0.22 [presumably the standard error] vs. 0.22 ± 0.03 in untreated males, 0.17 ± 0.02 in untreated females, 0.25 ± 0.05 in male vehicle controls, and 0.23 ± 0.04 female vehicle controls). Tumor multiplicity was not significantly increased in LD and MD mice.	Stoner et al. (1973; cited by IARC, 1990)
8-wk-old AKR mice	40F	40F (it was not specified if controls were untreated or were vehicle controls	5-AzaC, purity not specified	1.5 mg/kg bw (6.1 µmol/kg bw) injected i.p. 6 times over 20 days and then 0.8 mg/kg bw (3.3 µmol/kg bw) injected 6 times i.p. over the following 30 days (Dose was reduced after 20 days because of toxicity.) The vehicle was not specified.	50 days	This study was a screening assay. AKR mice are highly susceptible to leukemia. **Blood:** Positive (for leukemia) All 5-AzaC-treated mice had died of leukemia by 60 days. Leukemia was not detected in any of the controls during the observation period of 120 days and no control deaths were reported during this period..	Vesely and Cihák (1973; cited by IARC, 1990)

Table 4-1. Mammalian Carcinogenicity of Azacitidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
BALB/c/A Bom Nhg (BALB/c) and CBA/Ca HSU Nhg (CBA) mice (17 wk old at start of study)	50F (BALB/c) 50F (CBA)	50F (BALB/c) 50F (CBA) It was not clear if controls were untreated or were vehicle controls.	5-AzaC, purity not specified	1 mg/kg bw (4 μmol/kg bw) in saline or phosphate-buffered saline i.p. (injections at weekly intervals until wk 4, at 3 weekly intervals after wk 6; 11 injections over a 21-wk period)	21 wk (147 days)	A complete necropsy was performed on all dead and moribund-killed animals. Pathological diagnosis was confirmed by histological examination. Statistical analysis of tumor incidence was not performed. **Hematopoietic System:** Positive (for "lymphoblastic leukemia-like lesion"; only in CBA mice) At 161 days after the start of the injection series (14 days after the 11th injection of 5-AzaC) the first case of an unusual lymphoblastic leukemia-like lesion was observed in the CBA group. On days 163 and 164, the rate of occurrence increased suddenly with 29 mice dying or becoming moribund. Of the 5-AzaC-treated CBA mice that survived the treatment period, 34/47 (vs. 0/50 CBA controls) developed the lesion, the last case occurring on day 189. None of the 5-AzaC-treated BALB/c mice or BALB/c controls developed the lesion, nor did the surviving CBA mice over 150 succeeding days, suggesting an epigenetic mechanism of carcinogenesis.	Luz and Murray (1988)

Table 4-1. Mammalian Carcinogenicity of Azacitidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
8-wk-old BALB/c/Cb/Se mice	50M, 50F	50M, 50F (vehicle alone)	5-AzaC, 99% pure	2.0 mg/kg bw (8.2 µmol/kg bw) i.p. in saline, once/wk	50 wk	After 25 weeks of 5-AzaC treatment, survival was decreased in both sexes (no other details given). It was not specified in the IARC review which tissues were examined. The log rank test was used to analyze tumor incidence. **Lymphatic System:** Positive (for lymphoreticular neoplasia) The incidence of lymphoreticular neoplasms was significantly increased in 5-AzaC-treated mice (12/50 males vs. 3/50 controls [$p < 0.01$]; 36/50 females vs. 6/50 controls [$p < 0.001$]). **Lungs:** Positive (for adenoma; males only) The incidence of pulmonary adenoma was significantly increased in males, but not females, treated with 5-AzaC (27/50 vs. 12/50 controls [$p < 0.01$]; female incidence not listed). **Mammary Glands:** Positive (for adenocarcinoma and adenocanthoma*) Adenocarcinoma and adenocanthoma were detected in 7/50 5-AzaC-treated females (vs. none of controls). * IARC noted that the term "adenocanthoma" is not normally used to described mammary tumors. **Skin:** Positive (for tumorigenesis) The incidence of skin tumors (type not specified) was significantly increased in 5-AzaC-treated mice (3/50 males vs. 0/50 controls [$p < 0.05$]; 7/50 females vs. 1/50 controls [$p < 0.01$]).	Cavaliere et al. (1987; cited by IARC, 1990)

Table 4-1. Mammalian Carcinogenicity of Azacitidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
38-day-old B6C3F1 mice	35M, 35F per dose	15M, 15F (vehicle alone) 15M, 15F (untreated)	5-AzaC, > 99% pure	2.2 or 4.4 mg/kg bw (9.0 or 18 μmol/kg bw) in buffered saline, 3 times/wk	52 wk	Surviving mice were killed after 81 or 82 weeks. All HD females had died or been sacrificed before week 62. Seventeen of the LD females survived to the end of the study. Seven HD and 13 LD males survived to the end of the study. Twenty-five of the untreated controls (14M and 11F) and 20 vehicle controls (9M and 11F) survived to the end of the study. Tumor incidences were evaluated using Fisher's exact test, Cochran-Armitage test, and life-table methods as appropriate. **Hematopoietic System:** Positive (for lymphocytic and granulocytic neoplasia in females) The incidence of combined lymphocytic and granulocytic neoplasms of the hematopoietic system was significantly increased ($p < 0.001$) in LD females (17/29 vs. 0/14 vehicle controls and 1/15 untreated controls [malignant lymphocytic lymphoma]). LD females had 10 granulocytic tumors (9 sarcomas, 1 leukemia) and 7 malignant lymphomas. Early deaths in the HD group may have precluded the development of neoplasia. In LD and HD males, there was no increase in tumor incidence as compared to controls.	NCI 42 (1978); IARC (1990)

Table 4-1. Mammalian Carcinogenicity of Azacitidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - *Transplacental Administration*							
pregnant NMRI mice	32–37F per treatment	53F (vehicle alone)	5-AzaC, purity not specified	1 or 2 mg/kg bw (4 or 8 µmol/kg bw) i.p. in saline on day 12, 14, or 16 of gestation	not specified	The number of stillbirths was increased with the HD (value not given). Survival of offspring was decreased in all 5-AzaC-treated groups (details not given). Statistical analysis of tumor incidence was not mentioned in the IARC review. **Blood and Lymphatic System:** The combined incidence of leukemia and lymphoma was increased only in 1-mg offspring treated with 5-AzaC beginning on day 12 or day 16 of gestation and in 2-mg offspring treated with 5-AzaC beginning on day 16 of gestation (day 12: 81/165 1-mg males, 80/158 1-mg females, 28/113 2-mg males, 26/110 2-mg females; day 14: 42/178 1-mg males, 26/171 1-mg females, 9/97 2-mg males, 14/101 2-mg females; day 16: 97/153 1-mg males, 98/160 1-mg females, 67/158 2-mg males, 57/151 2-mg females [vs. 84/293 male controls and 82/279 female controls]). Incidences of leukemia and lymphoma were not listed separately. **Lungs:** The incidence of lung tumors (type not specified) was increased only in 1-mg offspring treated with 5-AzaC beginning on day 16 of gestation and in 2-mg offspring treated with 5-AzaC beginning on day 14 or 16 of gestation (day 12: 30/165 1-mg males, 33/158 1-mg females, 22/113 2-mg males, 22/110 2-mg females; day 14: 29/178 1-mg males, 31/171 1-mg females, 46/97 2-mg males, 43/101 2-mg females; day 16: 81/153 1-mg males, 99/160 1-mg females, 78/158 2-mg males, 82/151 2-mg females [vs. 57/293 male controls and 53/279 female controls]). **Liver:** The incidence of liver tumors (type not specified) was increased only in certain groups (day 12: 15/165 1-mg males, 11/113 2-mg males, 9/110 2-mg females; day 14: 12/178 1-mg males, 20/171 1-mg females, 11/97 2-mg males, 7/101 2-mg females; day 16: 14/153 1-mg males, 8/160 1-mg females, 18/158 2-mg males [vs. 14/293 male controls and 11/279 female controls]). **Other:** It was reported in the IARC review that "some increases in the incidence of soft-tissue sarcomas were also seen", but no details were given.	Schmahl et al. (1985; cited by IARC, 1990)

Table 4-1. Mammalian Carcinogenicity of Azacitidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Intraperitoneal Injection*							
Sprague-Dawley rats (M initially dosed at age 35 days; F, at 42 days)	35M, 35F per dose	15M, 15F (vehicle alone) 15M, 15F (untreated)	5-AzaC, > 99% pure	2.6 or 5.2 mg/kg bw (11 or 21 μmol/kg bw) 3 times/wk	34 wk	Surviving rats were killed at 80 or 81 weeks. Negative for tumorigenicity. 1 HD M and 3 HD F had tumors. None of the tumors occurring in LD rats was significantly increased as calculated by any statistical test. Treated rats of both sexes showed bone marrow atrophy. The short life span and short treatment periods due to high mortality in the HD groups precluded evaluation of the carcinogenicity of 5-AzaC in male and female rats.	NCI 42 (1978)
21-day-old F344 rats (25-g weanlings)	10M	50 M (saline alone i.p. 3 times/wk for 1 yr)	5-AzaC, purity not specified	5 mg/kg bw (20.5 μmol/kg bw) i.p. in saline [Regimen 12 in Tables I and II of the paper]	single dose	Rats were examined 1 year after birth. **All Examined Tissues:** Negative The incidences of testes tumors and non-testis tumors were "not significantly" increased.	Carr et al. (1988)
Fischer rat (age not specified; 160–180 g)	12M (LD) 8M (HD)	12M (untreated)	5-AzaC, purity not specified	2.5 or 10 mg/kg bw (10 or 41 μmol/kg bw) in saline twice/wk i.p.	9 mo	All rats were killed at 18 months. Statistical analysis of tumor incidence was not mentioned in the IARC review. **Testes:** Interstitial-cell tumors were detected in 9/12 LD and 1/8 HD rats (vs. 0/12 controls). IARC noted that although no testicular tumors were detected in controls in this study, testicular tumors occurred commonly in controls in a later study, conducted by the same authors, in which 5-AzaC was administered for 1 year (see below). **Skin:** Squamous cell carcinomas were detected in 2/8 HD rats, but in no other groups. **Injection Site:** A "skin appendage tumor at the site of injection" was detected in a HD male, but in no other groups. IARC noted the small number of animals used in this study.	Carr et al. (1984; cited by IARC, 1990)

Table 4-1. Mammalian Carcinogenicity of Azacitidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
F344 rats (young adults; 160-180 g)	10M (LD) 10M (MD) 100M (HD)	50M (age controls given saline i.p. 3x/wk for 1 yr)	5-AzaC, purity not specified	0.025, 0.25, or 2.5 mg/kg bw (0.1, 1.0 or 10 μmol/kg bw) in saline, 3 times/wk i.p. [Regimens 2-4 in Tables I and II of the paper]	1 yr	Rats were killed at the end of the 1-year treatment period, at which time all LD and MD, but only 87/100 HD, rats were alive. Fisher's exact test was used to evaluate tumor incidence. **Testes:** Positive (for interstitial-cell tumors) The incidence of interstitial-cell tumors was significantly increased in HD rats (56/87 vs. 10/49 controls; $p < 0.01$). The incidence in LD and MD rats was not significant. **Other:** No other tumors were detected in controls. In HD rats, other tumors detected were 4 lymphomas, 4 renal tumors, 1 lung tumor, 3 skin tumors, 2 mesotheliomas, and 2 sarcomas. No other details about these tumors were given. LD and MD rats did not develop any non-testis tumors.	Carr et al. (1988); IARC (1990)
Rats - *Transplacental Administration*							
pregnant F344 rats on day 21 of timed pregnancies	5F	50M (age controls given saline i.p. 3x/wk for 1 yr)	5-AzaC, purity not specified	10 mg/kg bw (41 μmol/kg bw) i.p. in saline [Regimen 11 in Tables I and II of the paper]	single dose	Offspring (13 males, 9 females) were examined 1 year after birth. **Testes:** Negative The incidence of tumors of the testes in male offspring was "not significant" (3/13 [23%] vs. 10/49 [20%]). **Other Tissues:** Positive The incidence of non-testis tumors was increased in offspring (3/22 [1 lymphoma, 1 lung tumor, 1 skin tumor; breakdown by sex not clear] vs. 0/49 in the controls; $p < 0.03$, Fisher's exact test).	Carr et al. (1988)

Abbreviations: bw = body weight; F = females; HD = high dose; i.p. = intraperitoneally; LD = low dose; M = males; MD = mid dose

5.0 GENOTOXICITY

Studies of the genotoxic effects of 5-AzaC are summarized in Table 5-1.

Summary: 5-AzaC was found to be genotoxic in a wide variety of prokaryotic (noneukaryotic), lower eukaryotic, and mammalian *in vitro* test systems [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1990)]. Unless otherwise stated, 5-AzaC was tested only in the absence of S9. 5-AzaC was found to induce DNA damage in *Escherichia coli* and HeLa cells; sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells and human lymphocytes; mutations in *E. coli, Salmonella typhimurium* (base pair substitution mutations only), *Saccharomyces cerevisiae* (mitotic recombination, gene conversion, and gene mutations), *Drosophila melanogaster* (somatic mutations, mitotic recombination, paternal chromosome loss and interchanges in immature germ cells), soybean plants, mouse lymphoma L5178Y cells (but not in the presence of metabolic activation), and human lymphocytes *(tk* locus only*);* chromosomal aberrations in *S. cerevisiae*, Chinese hamster DON cells, CHO cells, hamster embryo fibroblasts, rat pituitary tumor cells, and human lymphocytes; micronuclei (MN) in Syrian hamster embryo (SHE) and mouse lymphoma L5178Y cells; and morphological transformation in mouse C3H-10T1/2 and BALB/c-3T3 cells, hamster embryo fibroblasts, and rat primary tracheal epithelial cells. Treatment with 5-AzaC also affected the ability of ultraviolet (UV) light to induce mutations in *E. coli* and synergistically enhanced the ability of 9-aminoacridine to induce reverse mutations in *S. typhimurium.* It was negative for the induction of frameshift mutations in *S. typhimurium;* aneuploidy in yeast; DNA damage measured by unscheduled DNA synthesis (UDS) in SHE cells; mutations (ouabain resistance and at the *hprt* locus) in V79 cells, mouse lymphoma cells, C3H cells, Syrian hamster BHK cells, and primary rat tracheal epithelial cells; and chromosome aberrations in *Vicia faba* root tips. In the only *in vivo* study located, 5-AzaC was negative for dominant lethal mutations in mice. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro.*

Information for studies reviewed in IARC was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., generally not provided. Because of the extensive information available in IARC (1990) on the *in vitro* genotoxicity of 5-AzaC, the search strategy was limited to studies that offered unique information. In addition, for simplicity, multiple citations in IARC for the same genetic toxicity assay were discussed as a group rather than individually.

5.1 Noneukaryotic Systems

5.1.1 DNA Damage

Barbe et al. (1986; cited by IARC, 1990) reported that 5-AzaC induced lambda prophage in *E. coli* [LED = 40 μg/plate (0.16 μmol/plate)], while Bhagwat and Roberts (1987; cited by IARC, 1990) concluded that 5-AzaC induced DNA damage, as measured by the rec A assay, in *E. coli* [LED = 4.0 μg/plate (0.016 μmol/plate)].

5.1.2 Gene Mutations

IARC (1990) reviewed 2 papers in which 5-AzaC induced both forward (strain K12) and reverse (strain WP2) mutations in *E. coli* [LED = 0.2 μg/plate (0.0008 μmol/plate)]. In addition, Radnedge and Pinney (1993) evaluated the effect of 5-AzaC on UV light-induced mutations in a

series of repair-proficient and -deficient strains of *E. coli* and concluded that 5-AzaC may induce a normally error-free DNA repair pathway to become error-prone. IARC (1990) also cited 4 papers that stated 5-AzaC induced base-pair mutations in *S. typhimurium* strains TA100, TA92, TA102, TA104, TA1535, TA2638, TA2661, and TA4006 [LED = 4.8 μg/plate (0.025 μmol/plate)] but not frameshift mutations in strains TA98, TA96, TA97, TA1538, and TA2640 [HID = 50 μg/plate (0.20 μmol/plate)]. Podger and Grigg (1986) reported that 5-AzaC induced a synergistic enhancement in the frequency of frameshift mutations induced by 9-aminoacridine in *S. typhimurium* strain *his*C3076 [LED = 5 μg/plate (0.02 μmol/plate)].

5.2 Lower Eukaryotic Systems

Zimmerman and Scheel (1984; cited by IARC, 1990) reported that 5-AzaC in the absence of metabolic activation induced mitotic recombination [LED = 5000 μg/plate (20 μmol/plate)], mitotic gene conversion [LED = 2000 μg/plate (8.2 μmol/plate)], and reverse gene mutations [LED = 2000 μg/plate (8.2 μmol/plate)], but not aneuploidy [HID = 10,000 μg/plate (41 μmol/plate)] in *S. cerevisiae.* In *D. melanogaster,* Katz et al. (1985; cited by IARC, 1990) found that 5-AzaC induced mitotic recombination, deletions, and mutations in the wing spot assay [LED = 244 μg/mL (1000 μM)]. Vogel and Nivard (1993) reported that 5-AzaC (at 10,000 μM) administered in food induced a significant increase in mitotic recombination in the eye mosaic assay, while Osgood and Seward (1989) found that 5-AzaC (tested at 100 to 1,000 μg/mL; 409 to 4100 μM) in food induced sex chromosome loss (partial and complete) and interchanges in immature germ cells of *mei-9* males. In agreement, Pontecorvo et al. (1992) reported that 5-AzaC induced paternal chromosome recombination in immature germ cells of *D. melanogaster* Oregon-R and *mei-9* males. Fucik et al. (1970; cited by IARC, 1990) stated that chromosomal aberrations were not induced in the root meristem cells of *V. faba* treated with 5-AzaC [HID = 24 μg/mL (100 μM)]. 5-AzaC at 10 to 100 μg/mL (41 to 409 μM) was reported (Katoh et al., 1993) to induce a significant increase in mutational spots (considered to be due to somatic crossing over) on the leaves of soybean plants (strain T-219) produced from seeds treated for 24 h at 20°C in the dark [LED = 25 μg/mL (100 μM)].

5.3 Mammalian Systems *In Vitro*

5.3.1 DNA Damage

Snyder and Lachmann (1989; cited by IARC, 1990) reported that 5-AzaC induced DNA strand breaks (detection method not specified) in HeLa cells [LED = 48 μg/mL (200 μM)]. However, 5-AzaC was negative for UDS in Syrian hamster embryo (SHE) cells treated for 5 hours in the absence of metabolic activation at doses of 0.2 to 2.0 μM (Stopper et al., 1992). IARC, Vol. 50 (1990) reported on 2 papers stating that 5-AzaC induced SCE in CHO cells [LED = 0.24 μg/mL (1.0 μM)]. Perticone et al. (1987) and Perticone et al. (1990) reported also that 5-AzaC induced a significant increase in SCE in CHO cells (LED = 10 μM); however, it should be noted that the increase was stable across cell cycles (as long as 16) even in the absence of continued exposure to 5-AzaC. Lavia et al. (1985; cited by IARC, 1990) found that SCE were also induced in human peripheral blood lymphocytes treated with 8.0 μM 5-AzaC. All studies were conducted in the absence of S9.

5.3.2 Gene Mutations

Marquardt and Marquardt (1977; cited by IARC, 1990) reported that 5-AzaC without S9 induced ouabain-resistant mutants in Chinese hamster lung V79 cells [LED = 1 μg/mL (4 μM)]. However, this result was not reproduced by several subsequent studies. Landolph and Jones (1982; cited by IARC, 1990) concluded that 5-AzaC without S9 did not induce ouabain-resistant mutants [HID = 0.7 μg/mL (3 μM)] in V79 cells. Similarly, Bouck et al. (1982; cited by IARC, 1990) did not find 5-AzaC to induce ouabain-resistant mutants [HID = 2.4 μg/mL (10 μM)] in Syrian hamster BHK cells, nor did Landolph and Jones (1982; cited by IARC, 1990) for mouse C3H 10T1/2 cells without S9 activation [LED = 2.4 μg/mL (10 μM)]. Walker and Nettesheim (1986; cited by IARC, 1990) likewise reported that 5-AzaC failed to induce ouabain-resistant mutants in rat primary tracheal epithelial cells [HID = 1.0 μg/mL (4.0 μM)]. 5-AzaC was reported also as negative for mutations at the *hprt* locus by Landolph and Jones (1982; cited by IARC, 1990) for V79 cells [HID = 0.7 μg/mL (3 μM)], by Bouck et al. (1982; cited by IARC, 1990) in Syrian hamster BHK cells [HID = 2.4 μg/mL (10 μM)], and by McGregor et al. (1989; cited by IARC, 1990) in mouse lymphoma cells [HID = 0.33 μg/mL (1.4 μM)]. In contrast, Call et al. (1986; cited by IARC, 1990) reported that 5-AzaC without metabolic activation was positive for the induction of *hprt* mutations [LED = 0.12 μg/mL (0.50 μM)] in human peripheral blood lymphocytes. In contrast to the largely negative data for these two loci, Amacher and Turner (1987; cited by IARC, 1990) and McGregor et al. (1989; cited by IARC, 1990) reported increased mutations at the *tk* locus in mouse lymphoma L5178Y cells treated with 5-AzaC in the absence but not the presence of metabolic activation [LED = 0.02 μg/mL (0.08 μM) and LED = 0.01 μg/mL (0.04 μM), respectively]. Call et al. (1986; cited by IARC, 1990) concluded also that 5-AzaC without metabolic activation was positive for the induction of *tk* mutations [LED = 0.024 μg/mL (0.10 μM)] in human peripheral blood lymphocytes.

5.3.3 Chromosomal Damage

In the absence of metabolic activation, 5-AzaC has been reported to induce a significant increase in chromosomal aberrations in Chinese hamster Don cells [Karon and Benedict, 1972; cited by IARC, 1990; LED = 10 μg/mL (41 μM)], CHO cells [Benedict et al., 1977; cited by IARC, 1990; LED = 2.5 μg/mL (10 μM)], Chinese hamster embryo fibroblasts [Harrison et al., 1983; cited by IARC, 1990; LED = 0.73 μg/mL (3.0 μM)], and in human peripheral blood lymphocytes [Lavia et al., 1985; cited by IARC, 1990; LED = 1.95 μg/mL (8.0 μM)]. Investigators have speculated that the ability of incorporated 5-AzaC to impair chromosome condensation in genomic DNA may lead to an increased incidence of structural chromosome damage [e.g., mitogen-stimulated human lymphocytes (Schmid et al., 1984); rat pituitary tumor cells ($GH_1 2C_1$) (Parrow et al., 1989)]. In addition, 5-AzaC has been reported to induce micronuclei (MN) in SHE cells (LED = 1 μM; Stopper et al., 1992) and in mouse lymphoma L5178Y cells (LED = 0.1 μM [Stopper et al., 1993]; LED = 0.2 μM [Stopper et al., 1995]). In all three studies, kinetochore analysis revealed an increase predominantly in kinetochore-negative MN (presumably due to structural chromosomal damage) and, to a lesser extent, in kinetochore-positive MN (presumably due to numerical chromosomal damage).

5.3.4 Cell Transformation

In the absence of metabolic activation, 5-AzaC induced a significant increase in morphological transformation in C3H 10T1/2 [Benedict et al., 1977; cited by IARC, 1990; LED = 0.25 μg/mL (1.0 μM)], in Chinese hamster embryo fibroblasts [Harrison et al., 1983; cited by IARC, 1990; LED = 0.73 μg/mL (3.0 μM)], in primary rat tracheal epithelial cells [Walker and Nettesheim, 1986; cited by IARC, 1990; LED = 0.24 μg/mL (1.0 μM)], in mouse Balb/c 3T3 cells [Yasutake et al., 1987; cited by IARC, 1990; LED = 1.2 μg/mL (5.0 μM)], and in SHE cells [LED = 0.4 μM; Stopper et al., 1992). Walker and Nettesheim (1986) demonstrated that the 5-AzaC transformants were malignant upon injection into nude mice; however, the 5-AzaC-induced transformants did not demonstrate an increase in the expression of the H-*ras*, K-*ras*, or *raf* oncogenes.

5.4 Mammalian Systems *In Vivo*

Epstein et al. (1972; cited by IARC, 1990) reported that 5-AzaC at 5.0 and 10 mg/kg (20 and 41 μmol/kg) administered in a single i.p. dose did not induce dominant lethal mutations in male mice (strain not provided).

Table 5-1. Summary of Azacitidine Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Noneukaryotic Systems							
5.1.1 DNA Damage							
Escherichia coli (strains not provided)	lambda prophage induction	-	n.p.	n.g.	positive	LED = 40 μg/plate (0.16 μmol/plate)	Barbe et al. (1986; cited by IARC, 1990)
E. coli (strains not provided)	DNA damage (rec A assay)	-	n.p.	n.g.	positive	LED = 4.0 μg/plate (0.016 μmol/plate)	Bhagwat and Roberts (1987; cited by IARC, 1990)
5.1.2 Gene Mutations							
E. coli strains WP2, K12, and others (not specified)	forward and reverse gene mutations (loci not specified)	-	n.p.	n.g.	positive	LED = 0.2 μg/plate (0.0008 μmol/plate)	Fucik et al. (1965); Lal et al. (1988); both cited by IARC (1990)
E. coli (multiple repair-proficient and repair-deficient strains)	*his* reverse gene mutations	-	n.p.	0.2 – 20 μg/plate (0.0008 – 0.08 μmol/plate) prior to ultraviolet (UV) light treatment	affected UV-induced mutant frequency	LED varied with strain; differential response among the various strains suggested that 5-AzaC may induce a normally error-free DNA repair pathway to become error-prone	Radnedge and Pinney (1993)
Salmonella typhimurium strains TA100, TA92, TA102, TA104, TA1535, TA2638, TA2661, and TA4006	*his* reverse gene mutations	-	n.p.	n.g.	positive	Positive for base-pair substitutions only, tested only in the absence of metabolic activation [LED = 4.8 μg/plate (0.025 μmol/plate)]	Marquardt and Marquardt (1977); Levin and Ames (1986); Schmuck et al. (1986); all cited by IARC (1990)

Table 5-1. Summary of Azacitidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
S. typhimurium strains TA98, TA96, TA97, TA1538, and TA2640	*his* reverse gene mutations	-	n.p.	n.g.	negative	Negative in frameshift mutations [HID = 50 μg/plate (0.20 μmol/plate)]	Podger (1983); Levin and Ames (1986); Schmuck et al. (1986); all cited by IARC (1990)
S. typhimurium strain *his*C3076 [LED = 5 μg/plate (0.02 μmol/plate)]	*his* reverse gene mutations	-	n.p.	5 - 50 μg/plate (0.02 - 0.2 μmol/plate)]	synergistic enhancement	5-AzaC induced a synergistic enhancement in the frequency of frameshift mutations induced by 9-aminoacridine. LED = 5 μg/plate (0.02 μmol/plate)	Podger and Grigg (1986)
5.2 Lower Eukaryotic Systems							
Saccharomyces cerevisiae	mitotic recombination	-	n.p.	n.g.	positive	LED = 5000 μg/plate (20 μmol/plate)	Zimmerman and Scheel (1984; cited by IARC, 1990)
S. cerevisiae	mitotic gene conversion	-	n.p.	n.g.	positive	LED = 2000 μg/plate (8.2 μmol/plate)	Zimmerman and Scheel (1984; cited by IARC, 1990)
S. cerevisiae	reverse gene mutations	-	n.p.	n.g.	positive	LED = 2000 μg/plate (8.2 μmol/plate)	Zimmerman and Scheel (1984; cited by IARC, 1990)
S. cerevisiae	aneuploidy	-	n.p.	n.g.	negative	HID = 10,000 μg/plate (41 μmol/plate)	Zimmerman and Scheel (1984; cited by IARC, 1990)
Drosophila melanogaster	wing spot (somatic mutations, deletions, and recombination) assay	-	n.p.	n.g.	positive for all three endpoints	LED = 244 μg/mL (1000 μM)	Katz (1985; cited by IARC, 1990)
D. melanogaster	(white/white$^+$) eye mosaic assay, mitotic recombination	-	n.p.	10,000 μM administered to the surface of the food	positive	LED = 10,000 μM	Vogel and Nivard (1993)

Table 5-1. Summary of Azacitidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
D. melanogaster (strain *mei 9*)	paternal chromosome loss and interchanges in immature germ cells	-	n.p.	100 - 1000 μg/mL (409 - 4100 μM) administered in food	positive	LED = 1000 μg/mL (4100 μM); increase noted for partial and complete loss and interchanges of the paternal sex chromosome	Osgood and Seward (1989)
D. melanogaster (strains *Oregon-R* and *mei 9*)	paternal chromosome recombination in immature germ cells	-	n.p.	244 and 610 μg/mL (1000 and 2500 μM) administered in food	positive	LED = 244 μg/mL (1000 μM)	Pontecorvo et al. (1992)
Vicia faba root meristem cells	chromosome aberrations	-	n.p.	n.g.	negative	HID = 24 μg/mL (100 μM)	Fucik et al. (1970; cited by IARC, 1990)
soybean plants (strain T-219)	mutational spots on leaves	-	n.p.	10 to 100 μg/mL (41 to 409 μM)	positive	seeds treated for 24 h at 20°C in the dark. Increase in twin spots considered to be due to somatic crossing over [LED = 25 μg/mL (100 μM)].	Katoh et al. (1993)
5.3 Mammalian Systems *In Vitro*							
5.3.1 DNA Damage							
HeLa cells	DNA strand breaks (method not specified)	-	n.p.	n.g.	positive	LED = 48 μg/mL (200 μM)	Snyder and Lachmann (1989; cited by IARC, 1990)
Syrian hamster embryo (SHE) cells	unscheduled DNA synthesis (UDS)	NA	n.p.	0.2 - 2.0 μM	negative	HID = 2.0 μM	Stopper et al. (1992)
Chinese hamster ovary (CHO) cells	sister chromatid exchanges (SCE)	-	n.p.	n.g.	positive	LED = 0.24 μg/mL (1.0 μM)	Hori (1983); Banerjee and Benedict (1979); both cited by IARC (1990)
CHO cells	SCE	-	n.p.	10 - 30 μM for 12 h	positive	LED = 10 μM. Increase stable across 10 cell cycles in absence of exposure	Perticone et al. (1987)
CHO cells	SCE	-	n.p.	10 μM for 12 h	positive	Increase stable across 16 cell cycles in absence of exposure	Perticone et al. (1990)

Table 5-1. Summary of Azacitidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
human peripheral blood lymphocytes	SCE	-	n.p.	1.95 μg/mL (8.0 μM) (exposure time was not provided)	positive	None	Lavia et al. (1985; cited by IARC, 1990)
5.3.2 Gene Mutations							
Chinese hamster lung V79 cells	ouabain-resistant mutations	-	n.p.	n.g.	positive	LED = 1 μg/mL (4 μM)	Marquardt and Marquardt (1977; cited by IARC, 1990)
Chinese hamster lung V79 cells	ouabain-resistant mutations	-	n.p.	n.g.	negative	HID = 0.7 μg/mL (3 μM)	Landolph and Jones (1982; cited by IARC, 1990)
Syrian hamster BHK cells	ouabain-resistant mutations	-	n.p.	n.g.	negative	HID = 2.4 μg/mL (10 μM)	Bouck et al. (1984; cited by IARC, 1990)
mouse C3H 10T1/2 cells	ouabain resistant mutations	-	n.p.	n.g.	negative	HID = 2.4 μg/mL (10 μM)	Landolph and Jones (1982; cited by IARC, 1990)
primary rat tracheal epithelial cells	ouabain-resistant mutations	-	n.p.	n.g.	negative	HID = 1 μg/mL (4 μM)	Walker and Nettesheim (1986; cited by IARC, 1990)
Chinese hamster lung V79 cells	*hprt* gene mutations	-	n.p.	n.g.	negative	HID = 0.7 μg/mL (3 μM)	Landolph and Jones (1982; cited by IARC, 1990)
Syrian hamster BHK cells	*hprt* gene mutations	-	n.p.	n.g.	negative	HID = 2.4 μg/mL (10 μM)	Bouck et al. (1984; cited by IARC, 1990)
mouse lymphoma L5178Y cells	*hprt* gene mutations	-	n.p.	n.g.	negative	HID = 0.33 μg/mL (1.4 μM)	McGregor et al. (1989; cited by IARC, 1990)
human peripheral blood lymphocytes	*hprt* gene mutations	-	n.p.	n.g.	positive	LED = 0.12 μg/mL (0.50 μM)	Call et al. (1986; cited by IARC, 1990)

Table 5-1. Summary of Azacitidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
mouse lymphoma L5178Y cells	*tk* gene mutations	+/-	n.p.	n.g.	negative/ positive	-S9 LED = 0.02 μg/mL (0.08 μM)	Amacher and Turner (1987; cited by IARC, 1990)
mouse lymphoma L5178Y cells	*tk* gene mutations	-	n.p.	n.g.	positive	LED = 0.01 μg/mL (0.04 μM)	McGregor et al. (1989; cited by IARC, 1990)
human peripheral blood lymphocytes	*tk* gene mutations	-	n.p.	n.g.	positive	LED = 0.024 μg/mL (0.10 μM)	Call et al. (1986; cited by IARC, 1990)
5.3.3 Chromosomal Damage							
Chinese hamster DON cells	chromosome aberrations	-	n.p.	n.g.	positive	LED = 10 μg/mL (41 μM)	Karon and Benedict (1972; cited by IARC, 1990)
CHO cells	chromosome aberrations	-	n.p.	n.g.	positive	LED = 2.5 μg/mL (10 μM)	Benedict et al. (1977; cited by IARC, 1990)
Chinese hamster embryo fibroblasts	chromosome aberrations	-	n.p.	n.g.	positive	LED = 0.73 μg/mL (3.0 μM)	Harrison et al. (1983; cited by IARC, 1990)
human peripheral blood lymphocytes	chromosome aberrations	-	n.p.	1.95 μg/mL (8.0 μM) exposure time was not provided	positive	None	Lavia et al. (1985; cited by IARC, 1990)
human peripheral blood lymphocytes	chromosome aberrations	-	n.p.	0.3 μM for 7 h prior to fixation	positive	Treatment induced chromosome decondensation and breakage	Schmid et al. (1984)
rat pituitary tumor cells ($GH_1 2C_1$)	chromosome aberrations	-	n.p.	3 μM for 6 days	positive	Chromosome decondensation and breakage induced in subcultured cells following treatment	Parrow et al. (1989)

Table 5-1. Summary of Azacitidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
Syrian hamster embryo (SHE) cells	micronuclei (MN) induction	NA	n.p.	1 - 10 μM for 5 h	positive	LED = 1 μM; kinetochore analysis indicated MN arising from both structural and numerical chromosome damage (structural > numerical)	Stopper et al. (1992)
mouse lymphoma L5178Y cells	MN induction	-	n.p.	0.1 - 5 μM for 4 h	positive	LED = 0.1 μM; kinetochore analysis indicated MN arising from both structural and numerical chromosome damage (structural > numerical)	Stopper et al., (1993)
mouse lymphoma L5178Y cells	MN induction	-	n.p.	0.05 - 1.0 μM for 4 h	positive	LED = 0.2 μM; kinetochore analysis indicated MN arising from both structural and numerical chromosome damage (structural > numerical)	Stopper et al., (1995)
5.3.4 Cell Transformation							
C3H 10T1/2 mouse cells	morphological transformation	NA	n.p.	n.g.	positive	LED = 0.25 μg/mL (1.0 μM)	Benedict et al. (1977; cited by IARC, 1990)
Chinese hamster embryo fibroblasts	morphological transformation	NA	n.p.	n.g.	positive	LED = 0.73 μg/mL (3.0 μM)	Harrison et al. (1983; cited by IARC, 1990)
rat primary tracheal epithelial cells	morphological transformation	NA	n.p.	n.g.	positive	LED = 0.24 μg/mL (1.0 μM); transformants were malignant upon injection into nude mice; however, the 5-AzaC-induced transformants did not demonstrate an increase in the expression of the H-*ras*, K-*ras*, or *raf* oncogenes.	Walker and Nettesheim (1986; cited by IARC, 1990)
BALB/c 3T3 mouse cells	morphological transformation	NA	n.p.	n.g.	positive	LED = 1.2 μg/mL (5.0 μM)	Yasutake et al. (1987; cited by IARC, 1990)
SHE cells	morphological transformation	NA	n.p.	0.2 - 2.0 μM for 5 h	positive	LED = 0.4 μM	Stopper et al. (1992)

Table 5-1. Summary of Azacitidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.4 Mammalian Systems *In Vivo*							
5.4.1 Gene Mutations							
male mice (species not provided)	dominant lethal mutations	NA	n.p.	5.0 and 10 mg/kg bw (20 and 41 μmol/kg) i.p.	negative	HID = 10 mg/kg bw (41 μmol/kg)	Epstein et al. (1972; cited by IARC, 1990)

Abbreviations: HID = highest ineffective dose; i.p. = intraperitoneally; LED = lowest effective dose; NA = not applicable; n.g. = not given; n.p. = not provided

Figure 5-1. Genetic Activity Profile of Azacitidine (Data limited to IARC, 1990)

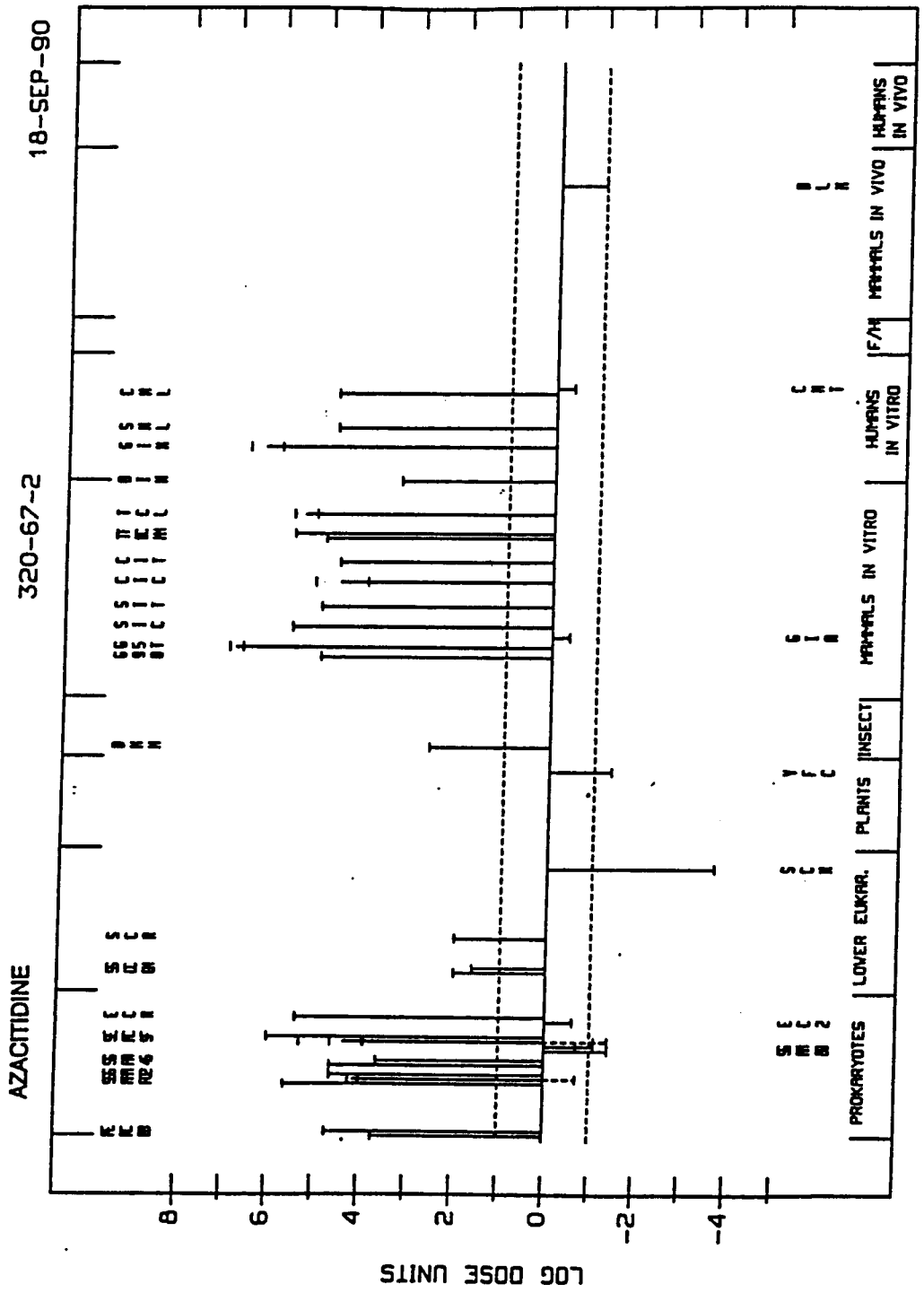
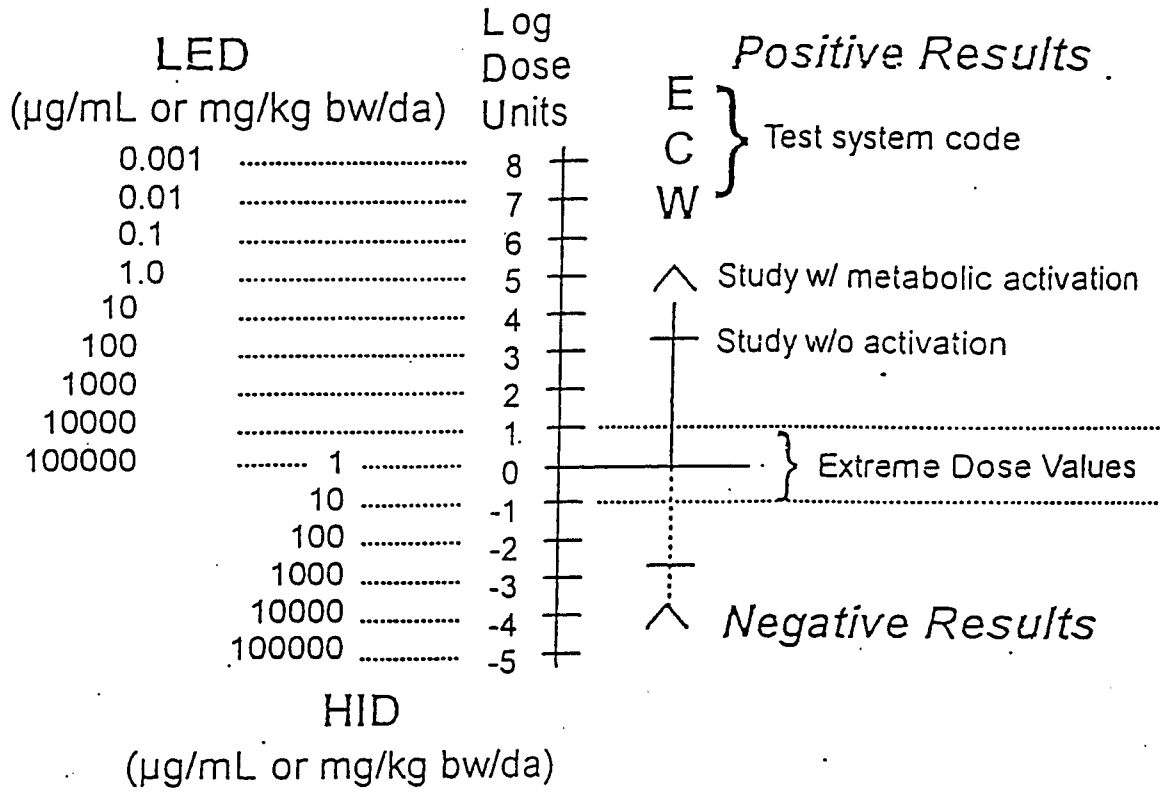


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT DATA

6.1 Absorption, Distribution, Metabolism, and Excretion

Summary: Metabolism and distribution of 5-AzaC are similar in humans, mice, and dogs. Enzymes involved in metabolism include cytidine deaminase and uridine-cytidine kinase. Metabolites include 5-azacytidine triphosphate, 5-azacytosine, 5-azauracil, 5-azauridine, guanidine-related products, D-ribose, and urea.

The absorption, distribution, metabolism, and excretion of 5-AzaC are summarized in Table 6-1 by biological systems. Metabolites and decomposition products are summarized in Table 6-2. Chemical decomposition pathways are depicted in Figure 6-1. 5-AzaC is a structural analog of the nucleoside cytidine (cytosine riboside). In 5-AzaC, the carbon atom at the 5 position of the cytosine ring has been replaced by a nitrogen atom.

The reported plasma half-life ($t_{1/2}$) for 5-AzaC after i.p. or s.c. injection in mice and humans ranged from 2.5 to 5.4 h. The metabolism and disposition appear to be similar in humans, mice, and dogs. It was rapidly eliminated in the urine with similar excretion patterns (von Hoff and Slavik, 1977). Vogler et al. (1974) pointed out that the maximum tolerated doses are different. Humans tolerate relatively higher doses than rodents and dogs, which may be attributed to the relative activity of kinases and deaminases among the species.

The rapid *in vitro* chemical decomposition of 5-AzaC in aqueous solutions was described by Pithova et al. (1965; cited by von Hoff and Slavik, 1977). See Figure 6-1. In neutral and basic media, 5-AzaC is hydrolyzed to 1-β-ribofuranosyl-3-guanylurea (III in Figure 6-1) (probably the same compound as guanylurea ribonucleoside) via oxidative degradation of the bond between the carbon atom at the 6 position of the cytosine ring and the nitrogen atom at position 1 accompanied by loss of that carbon atom as formaldehyde or formate. Further degradation was reported to give α-D-ribofuro(1′,2′:4,5)-2-azolidone, guanidine, and D-ribose. At 37 °C and pH 7.2, the concentration of 5-AzaC underwent an initial rapid decline, but approximately 25% of the 5-AzaC was unchanged after 24 h.

In acidic media, 5-AzaC underwent deglycosylation (loss of the ribose moiety) to give 5-azacytosine (V in Figure 6-1). Oxidative deamination of 5-azacytosine gave 5-azauracil (VI). Deamination occurring before deglycosylation led to 5-azauridine (ribosyl derivative of VI) [Pithova et al., 1965; cited by von Hoff and Slavik, 1977].

Enzymic reactions were not identified for most of the compounds in Figure 6-1. The deamination reactions are catalyzed by cytidine deaminase. The intracellular phosphorylation to 5-AzaC triphosphate is catalyzed by uridine-cytidine kinase (Cihák, 1974; cited by IARC, 1990; Neil et al., 1975; cited by IARC, 1990; Israili et al., 1976; Plagemann et al., 1978; Glover and Leyland-Jones, 1987; Glover et al., 1987).

6.2 Pharmacokinetics

No information relevant to differences in pharmacokinetics among humans and rodents was found.

6.3 Mechanistic Information

Summary: 5-AzaC may induce carcinogenesis by mechanisms involved in its genotoxicity and/or its ability to inhibit DNA methylation.

The carcinogenic/promotional activity of 5-AzaC (see Sections 4.0 and 6.6) has been postulated to result directly or indirectly from its ability to inhibit DNA methylation (Harrison et al., 1983; for reviews, see Kerbel et al., 1984; Kerbel et al., 1986; Takenaga, 1986; Glover et al., 1987; Glover and Leyland-Jones, 1987; Jones and Buckley, 1990; Haaf, 1995), although Carr et al. (1988) concluded that there was no clear correlation between the level of DNA hypomethylation induced by several cytidine analogs and their carcinogenic activity. The first steps in this pathway (see review by Haaf, 1995) are thought to involve the transport of 5-AzaC into cells via the nucleoside transport system for uridine and cytidine and its subsequent activation to azacytidine triphosphate catalyzed by uridine-cytidine kinase. Azacytidine triphosphate is then incorporated into DNA, forming a base-pair with guanine. Once incorporated into DNA, 5-AzaC has been proposed to inhibit DNA methyltransferase noncompetitively by covalently binding to the enzyme. The irreversible binding of methyltransferase is thought to inhibit the methylation of cytosine residues downstream from the inactivated enzyme complex. An alternative mechanism in which the incorporation of 5-AzaC into DNA simply reduces the ability of DNA to serve as a methyl acceptor is not likely since it has been reported that the incorporation of only a small amount (5%) of 5-AzaC into DNA caused the almost complete (85-95%) demethylation of the DNA (see Haaf, 1995).

Altered levels of DNA methylation can affect gene expression (for reviews see Cedar, 1988; IARC, 1990; Fajkus et al., 1992; Velge et al., 1995), with hypomethylation being associated with the expression of genes that are normally silent or downregulated (Jones et al., 1983; Nyce et al., 1983; Riggs and Jones, 1983; Collard et al., 1989; Jones and Buckley, 1990; Pascale et al., 1993). If any of these overexpressed genes are growth regulators, their overexpression would likely increase tumorigenic potential. Consistent with this hypothesis, treatment of noninvasive BW5147 T-lymphoma cells *in vitro* with 5-AzaC resulted in invasive and metastatic cells that exhibited persistent genomic hypomethylation (Collard et al., 1989; Habets et al., 1990).

In addition to or because of its effect on DNA methylation, incorporated 5-AzaC is genotoxic in a number of prokaryote and eukaryote test systems (see Section 5.0). In lower eukaryote systems, the spectrum of genotoxic effects include mitotic recombination, gene conversions, mutations, and structural chromosomal damage. In cultured mammalian cells, induction of DNA damage (strand breaks/alkali-labile sites) and SCE, gene mutations, structural chromosomal aberrations, micronuclei, and morphological transformation were reported. Furthermore, some of these effects, such as an increased frequency of SCE in treated mammalian cells appears to be stable across multiple generations, even in the absence of continued treatment (Perticone et al., 1987; 1990). In at least one study (Stopper et al., 1993), it was demonstrated that the induction of micronuclei depended on the incorporation of 5-AzaC into DNA. It is hypothesized that these effects may relate to the well-established ability of incorporated 5-AzaC to interfere with normal chromosome condensation (reviewed in Haaf, 1995). Whether the genotoxic effects are due to incorporated 5-AzaC only or to the hypomethylated state induced by incorporated 5-AzaC remains to be determined. It should be noted, however, that 5-AzaC

induces a broad spectrum of genotoxic effects in Drosophila, an organism deficient in methylated DNA (Pontecorvo et al., 1992).

There are no data available that would suggest that the mechanisms thought to account for tumor induction by 5-AzaC in animals would not also operate in humans.

6.4 Structure-Activity Relationships

Carr et al. (1988) investigated 5-AzaC and five of its analogs (6-azacytidine, 5-aza-2′-deoxyazacitidine, 5-fluorodeoxycytidine, 5-fluorocytidine, and 5,6-dihydro-5-azacytidine) for carcinogenicity and their effect on hemoglobin gene expression (toxicity was also assessed). Only 5-AzaC proved to be a complete carcinogen in rats (see Section 4.0 for details), although it was noted that the sample size was small in bioassays for the other cytidine analogs. 5-AzaC was also able to induce minor hemoglobin synthesis in rats and mice (an indication that 5-AzaC is capable of gene activation); 5,6-dihydro-5-azacytidine and 5-aza-2′-deoxyazacitidine also had this ability. Based on these data and data from other studies that indicated that there may be a correlation between DNA inhibition and gene activation, the authors concluded that there is "no clear relationship among cytidine analogs between potency as inhibitors of DNA methylation and carcinogenic activity".

6.5 Cell Proliferation

Experimental details for the studies described in this section are presented in Table 6-3.

Summary: There was an increase in the incidence of Leydig cell hyperplasia in rats administered a single dose of 5-AzaC (5 mg/kg bw [20.5 μmol/kg bw]) i.p. at 21 days of age (25-g weanlings) and examined 1 year after birth (Carr et al., 1988). There was no significant increase in the incidence of Leydig cell hyperplasia, however, in young adult rats (160—180 g) administered 5-AzaC (2.5 mg/kg bw [10 μmol/kg bw]) i.p. 3 times/week for 1 year. When lower doses of 5-AzaC (0.025 or 0.25 mg/kg bw [0.1 or 1.0 μmol/kg bw]) were similarly administered, Leydig cell hyperplasia was not detected in any of the dosed rats (Carr et al., 1988; IARC, 1990). The labeling, synthetic, and mitotic indices were increased in the livers of Wistar rats administered 5-AzaC (5 μmol/100 g/day [12 mg/kg/day]) i.p. for 3 or 4 consecutive days. There was no change, however, in the level of thymidine-kinase or thymidylate-kinase in 5-AzaC-treated rats as compared to controls (Čihák and Seifertová, 1976). There was a slight increase in the incidence of Leydig cell hyperplasia in the offspring of female F344 rats administered a single dose of 5-AzaC (10 mg/kg bw [41 μmol/kg bw]) i.p. on day 21 of timed pregnancies. Statistical analysis, however, was not performed (Carr et al., 1988).

6.5.1 Intraperitoneal Injection

There was an increase in the incidence of Leydig cell hyperplasia in male F344 rats (25-g weanlings; females not evaluated) administered a single dose of 5-AzaC (5 mg/kg bw [20.5 μmol/kg bw]) i.p. in saline at 21 days of age and examined 1 year after birth (Carr et al., 1988).

There was no significant increase in the incidence of Leydig cell hyperplasia in young adult male F344 rats (160—180 g; females not evaluated) administered 5-AzaC (2.5 mg/kg bw [10 μmol/kg bw]) i.p. in saline 3 times/week for 1 year. When lower doses of 5-AzaC (0.025 or

0.25 mg/kg bw [0.1 or 1.0 μmol/kg bw]) were similarly administered, Leydig cell hyperplasia was not detected in any of the dosed rats (Carr et al., 1988; IARC, 1990).

The labeling, synthetic, and mitotic indices were increased in the livers of female Wistar rats (160-165 g; males not evaluated) administered 5-AzaC (5 μmol/100 g/day [12 mg/kg/day]) i.p. in saline for 3 or 4 consecutive days. There was no change, however, in the level of thymidine-kinase or thymidylate-kinase in 5-AzaC-treated rats as compared to controls (Čihák and Seifertová, 1976).

6.5.2 Transplacental Administration

There was a slight increase in the incidence of Leydig cell hyperplasia in the offspring of female F344 rats administered a single dose of 5-AzaC (10 mg/kg bw [41 μmol/kg bw]) i.p. in saline on day 21 of timed pregnancies (3/22 [13.6%] vs. 6/49 controls [12.2%]). Statistical analysis, however, was not performed (Carr et al., 1988).

6.6 Initiation/Promotion

Experimental details for the studies described in this section are presented in Tables 6-4 (carcinogenesis) and 6-5 (cell proliferation).

Summary: In outbred Syrian golden hamsters (sex not specified) administered 10% benzo[*a*]pyrene (B[*a*]P) in sustained released implants in the right lower lobe bronchus via tracheostomy and then 5-AzaC i.p. for up to 70 weeks, "the SPC [sequential progression of carcinogenesis] was slower in 5-AzaC-treated hamsters and the size of the cancers that developed was significantly less than in the controls [that received B[*a*]P alone]". In inbred (syngeneic) Syrian golden hamsters (sex not specified) similarly administered B[*a*]P, but administered 5-AzaC i.p. twice/week at varying times thereafter (group 1 received 5-AzaC continuously after B[*a*]P treatment, group 2 received 5-AzaC for only 80 days following B[*a*]P treatment, and group 3 received 5-AzaC beginning 80 days after B[*a*]P treatment and continuing throughout rest of experiment) there was no difference in any of the measured parameters (see above) between group 3 and the controls (B[*a*]P alone) and between groups 1 and 2. In groups 1 and 2, however, most parameters were significantly different from group 3 and the control group: The rate of SPC was slower, the cancers were smaller and better differentiated, and there were fewer nonepidermoid cancers in the hamsters in groups 1 and 2 than in the hamsters in group 3 and the control group

Treatment of male F344 rats administered *N*-nitrosodiethylamine (DEN; i.p. 18 hours after partial hepatectomy) with 5-AzaC i.p. twice/week for 9 months synergistically increased the frequency of liver tumors (type not specified) at 18 months. Also, 5-azaC (i.p. 3x a week for 1 year) enhanced the incidence lung and skin tumors in male F344 rats administered DEN administered i.p. 18 hours after partial hepatectomy.

5-AzaC in combination with a goitrogenic regime (0.2% aminotriazole, 0.5% sodium perchlorate, and 0.5% sucrose in drinking water from 28 days of age until 510 days of age), enhanced the incidence of thyroid tumors in female C3H x GPDX mice.

5-AzaC, administered i.p. as a single dose after B[*a*]P, *N*-methyl-*N*-nitrosourea (MNU), or 1,2-dimethylhydrazine (DMH), caused an increase in the incidence of initiated hepatocytes in F344 male rats.

6.6.1 Benzo[*a*]pyrene and 5-AzaC

In outbred Syrian golden hamsters (age and sex not specified) administered 10% benzo[*a*]pyrene (B[*a*]P) in sustained released implants in the right lower lobe bronchus via tracheostomy and then 5-AzaC (5 mg/kg [20 μmol/kg] twice/week) i.p. for up to 70 weeks, "the SPC [sequential progression of carcinogenesis] was slower in 5-AzaC-treated hamsters and the size of the cancers that developed was significantly less than in the controls [that received B[a]P alone]" (Hammond and Benfield, 1993).

In another experiment, inbred (syngeneic) Syrian golden hamsters (age and sex not specified) were similarly administered B[*a*]P, but administered 5-AzaC (5 mg/kg [20 μmol/kg] i.p. twice/week) at varying times thereafter. Group 1 received 5-AzaC continuously after B[*a*]P treatment, group 2 received 5-AzaC for only 80 days following B[*a*]P treatment, and group 3 received 5-AzaC beginning 80 days after B[*a*]P treatment and continuing throughout rest of experiment. Controls received B[*a*]P alone. There was no difference in any of the measured parameters (see above) between group 3 and the controls and between groups 1 and 2. In groups 1 and 2, however, most parameters were significantly different from group 3 and the control group: The rate of SPC was slower, the cancers were smaller and better differentiated, and there were fewer nonepidermoid cancers in the hamsters in groups 1 and 2 than in the hamsters in group 3 and the control group (Hammond and Benfield, 1993).

6.6.2 *N*-Nitrosodiethylamine and 5-AzaC

In male F344 rats administered *N*-nitrosodiethylamine (DEN; 50 mg/kg bw i.p. 18 hours after partial hepatectomy) in combination with 5-AzaC (2.5 or 10 mg/kg bw [10 or 41 μmol/kg bw] i.p. twice/week for 9 months) and killed at 18 months, liver tumors (type not specified) were detected in 2/10 low-dose and 8/10 high-dose rats, but not in the DEN controls (number not given). Although it appeared that DEN was given at the same time as 5-AzaC, it was not clear whether DEN was administered as a single dose or continuously over the 9-month 5-AzaC-treatment period (Carr et al., 1984; cited by IARC, 1990).

The incidence of non-testis tumors (lung and skin tumors) was significantly increased in 100-160 g male F344 rats administered DEN in combination with 5-AzaC in saline as compared to saline controls (no DEN controls were used). DEN (30 mg/kg [single dose]) was administered i.p. 18 hours after partial hepatectomy. 5-AzaC (2.5 mg/kg bw [8.2 μmol/kg bw]) was administered i.p. 3 times per week for 1 year. It was not clear if 5-AzaC administration was begun immediately after DEN administration. There was no significant increase in the incidence of testes tumors (Carr et al., 1988).

6.6.3 Goitrogen Treatment and/or ^{131}I and 5-AzaC

In a study conducted by Thomas and Williams (1992), C3H × GPDX female mice received 1 of 4 treatments: A goitrogenic regime (0.2% aminotriazole, 0.5% sodium perchlorate, and 0.5% sucrose in drinking water from 28 days of age until 510 days of age) in combination with either 5-AzaC or 5-aza-2-deoxycytidine (2.5 mg/kg [10 μmol/kg] i.p. in saline on days 5, 10, and 15 after start of the goitrogenic regime), a single i.p. injection of ^{131}I 7 days before the start of the goitrogenic regime, or the goitrogenic regime alone (controls). The incidence of thyroid tumors was increased in all groups as compared to the control group. In one mouse treated with the goitrogenic regime and 5-AzaC, a "very large carcinoma, occupying virtually the

whole of one thyroid lobe" was detected. This tumor metastasized to the lungs and was positive for thyroglobulin on immunohistochemistry. The authors noted that in previous studies, they did not detect carcinoma in over 100 mice administered goitrogen but no mutagen.

In another experiment conducted by Thomas and Williams (1992), C3H × GPDX female mice received 1 of 4 treatments: A single i.p. injection of ^{131}I at 21 days of age and either 5-AzaC or 5-aza-2-deoxycytidine (2.5 mg/kg [10 μmol/kg] i.p. in saline) at 33, 38, and 43 days of age (groups 1 and 2, respectively), or a single i.p. injection of ^{131}I at 21 days of age, goitrogen regime (see above) staring at 28 days of age, and either 5-aza-2-deoxycytidine or 5-AzaC at 33, 38, and 43 days of age (groups 3 and 4, respectively). Thyroid tumors were detected in all groups of mice except those in group 1, but statistical analysis was not performed to determine significance.

6.6.4 Initiated Hepatocytes and 5-AzaC

In F344 male rats (150-180 g), 5-AzaC, administered i.p. as a single dose (10 mg/kg [41 μmol/kg]) after MNU, or DMH, caused an increase in the incidence of initiated hepatocytes (number γ-GT positive foci/cm^2). 5-AzaC was administered 4 or 12 hours following a single i.p. injection of one of the carcinogens. The study was designed so that carcinogens were administered "at a dose which induced very few initiated hepatocytes." 5-AzaC was then administered during the phase of DNA repair synthesis "to facilitate its incorporation into DNA and thereby create stretches of hypomethylated DNA" (Denda et al., 1985).

Table 6-1. Absorption, Distribution, Metabolism, and Excretion of Azacitidine

Species	Dose	Route	Peak Concn. in Blood	Time to Peak or Half-Time	Distribution	Elimination	Metabolism	Reference(s)
Tumor cells					Transport into cells is by the "facilitated nucleoside transport system" for uridine and cytidine.		Phosphorylation catalyzed by uridine-cytidine kinase to 5-AzaC triphosphate, presumably the species incorporated into DNA and RNA of treated cells. Phosphorylation is rapid, saturable, and subject to potent feedback inhibition by uridine and cytidine triphosphates.	Li et al. (1970); Lee et al. (1975); both cited by Haaf (1995); Glover and Leyland-Jones (1987); Plagemann et al. (1978);
Mice	50 mg/kg bw	i.p.	43 μg/mL	≤0.5 h				Neil et al. (1975; cited by IARC, 1990)
Mice	9.5 mg/kg bw (LD_{10}) or 4.75 mg/kg bw	i.p.	n.g.	≤0.25 h	Not detected in liver, lung, brain, spleen, or kidneys at any time.	Rapid elimination. Not detected in blood after 1 h at the high dose or after 0.5 h at the low dose.		Pittello and Woolley (1969; cited by IARC, 1990)
Mice (AKR)	n.g. (radiolabeled)	i.p.	n.g.	$t_{1/2}$ = 3.8 h for AzaC and reactive metabolites	Radioactivity retained in lymphatic organs. Concn. in spleen and thymus higher at later intervals. Slight penetration into CNS.	^{14}C activity in blood diminished rapidly during the first 8 h but was still present after 24 h	Metabolites identified: Guanidine, 5-azauracil, biuret, 1-formylbiuret, and guanylurea ribonucleoside (presumably III in Figure 6-1)	Raska et al. (1965; cited by IARC, 1990, and by von Hoff and Slavik, 1977)
Mice	50 mg/kg bw	oral	2 μg/mL	≤0.5 h				Neil et al. (1975; cited by IARC, 1990)
Mice	n.g. in original abstract	n.g.				50% of the radiolabeled dose was excreted in urine within 8 h	Approx. 4% unchanged 5-AzaC plus 6 labeled metabolites were found in urine.	Coles et al. (1975 abstr.; cited by IARC, 1990)

Table 6-1. Absorption, Distribution, Metabolism, and Excretion of Azacitidine (Continued)

Species	Dose	Route	Peak Concn. in Blood	Time to Peak or Half-Time	Distribution	Elimination	Metabolism	Reference
Rabbits	15 mg/kg bw	i.v.				25-40% of the dose was excreted in urine and small amts. were excreted in the bile. Time elapsed was not given in IARC.		Chen et al. (1977; cited by IARC, 1990)
Beagle dogs	0.5 mg/kg bw	i.v.				33% of the dose was excreted in urine within 4 h.	Unchanged 5-AzaC was found in the urine plus the metabolites 5-azacytosine, urea, and guanidine.	Coles et al. (1974; cited by IARC, 1990)
Human	1.6 mg/kg bw	i.v.		$t_{1/2}$ = 3.8 h				Troetel et al. (1972; cited by von Hoff and Slavik, 1977)
Human	1.6 mg/kg bw	s.c.		< 5 h; $t_{1/2}$ = 4.2 h				Troetel et al. (1972; cited by von Hoff and Slavik, 1977)
Human	50 mg/m^2 by continuous infusion	i.v.	0.3 to 0.5 μg/mL (~1 to 2 mM) in plasma	$t_{1/2}$ = 2.5 to 5.4 h	Leukocyte/plasma ratio was 1.1 to 2.3. Less than 1% bound to human serum albumin *in vitro*.	α-phase half-time of radio-activity was 16 to 33 min; β-phase..., 3.4 to 6.2 h. 73-98% of injected label was eliminated in urine within 72 h Most label was eliminated in urine with small amts. In feces, sputum, and vomitus. The small amts. in vomitus suggested gastric and/or biliary secretion of 5-AzaC and/or its metabolites.	Within 0.5 h, only 2% unchanged 5-AzaC was found in urine. TLC detected ≥ 2 metabolites/decompn. products (5-azacytosine and 5-azauracil). *In vitro*, the $t_{1/2}$ of 5-AzaC in human urine at pH 6.5 and 25 °C was 70 h. *In vitro*, the $t_{1/2}$ in human plasma at pH 7.4 and 25 °C was 70 h. Max. nonenzymatic decompn. probably accounts for only ~20% of the overall decrease of the drug in plasma and tissues after 0.5 h.	Israili et al. (1976); IARC (1990)

Table 6-1. Absorption, Distribution, Metabolism, and Excretion of Azacitidine (Continued)

Species	Dose	Route	Peak Concn. in Blood	Time to Peak or Half-Time	Distribution	Elimination	Metabolism	Reference
Human	200 mg/m^2	n.g.		$t_{1/2}$ = 3 to 4.7 h		Almost all of the radioactivity was gone by 24 to 48 h. Approx. 90% excreted in the urine within 24 h. No significant amts. found in sputum, feces, or vomitus.		Vogler et al. (1974; cited by von Hoff and Slavik, 1977)

Abbreviations: i.p. = intraperitoneally; i.v. = intravenously; n.g. = not given; s.c. = subcutaneously

Table 6-2. Azacitidine Metabolites and Decomposition Products

Metabolite/Decompn. Product [CASRN]	Class of Reaction	Species or System	Reference
5-Azacytidine triphosphate	Sequential intracellular phosphorylation catalyzed by uridine-cytidine kinase	Mammalian cells (e.g., cultured Novikoff rat hepatoma and P388 mouse leukemia cells)	Plagemann et al. (1978); Glover and Leyland-Jones (1987); Glover et al. (1987); Haaf (1995)
5-Azacytosine (931-86-2) (V in Figure 6-1)	Hydrolysis under acidic conditions; deglycosylation	Female beagle dog, human	Pithova et al. (1965; cited by von Hoff and Slavik, 1977); Coles et al. (1974; cited by IARC, 1990); Israili et al. (1976)
5-Azauracil (VI in Figure 6-1)	Hydrolysis under acidic conditions; deglycosylation and deamination	AKR mouse, female beagle dog, human	Pithova et al. (1965; cited by von Hoff and Slavik, 1977); Raska et al. (1965; cited by von Hoff and Slavik, 1977); Coles et al. (1974; cited by von Hoff and Slavik, 1977); Israili et al. (1976)
5-Azauridine (ribosyl derivative of VI)	Deamination catalyzed by cytidine deaminase. Process inhibited by enzyme hydrouridine	"Biological systems," human	Cihák (1974; cited by IARC, 1990); Neil et al. (1975; cited by IARC, 1990); Israili et al. (1976); Glover and Leyland-Jones (1987)
Biuret [108-19-0] and 1-Formylbiuret	Decomposition of 5-azauracil (VI)	AKR mouse	Raska et al. (1965; cited by Von Hoff and Slavik, 1977)
Guanidine or guanidine-related products	Hydrolysis under neutral or basic conditions	AKR mouse, beagle dog, rat, and human plasma	Pithova et al. (1965; cited by von Hoff and Slavik, 1977); Raska et al. (1965; cited by von Hoff and Slavik, 1977); Coles et al. (1974; cited by von Hoff and Slavik, 1977); Glover et al. (1987)
1-β-Ribofuranosyl-3-guanylurea; Guanylurea ribonucleoside (III in Figure 6-1)	Hydrolysis under neutral or basic condition	AKR mouse	Raska et al. (1965; cited by Von Hoff and Slavik, 1977)
α-D-Ribofuro(1',2':4,5)-2-azolidone	Hydrolysis under neutral or basic conditions		Pithova et al. (1965; cited by Von Hoff and Slavik, 1977)
D-Ribose [50-69-1]	Hydrolysis under neutral, basic, or acidic conditions		Pithova et al. (1965; cited by Von Hoff and Slavik, 1977)
Urea [57-13-6]	Decomposition	Female beagle dog, human plasma	Coles et al. (1974; cited by IARC, 1990); Israili et al. (1976)

Table 6-3. Cell Proliferation Induced by Azacitidine

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Intraperitoneal Injection*							
21-day-old F344 rats (25-g weanlings)	10M	50 M (saline alone i.p. 3 times/wk for 1 yr)	5-AzaC, purity not specified	5 mg/kg bw (20.5 µmol/kg bw) i.p. in saline [Regimen 12 in Tables I and II of the paper]	single dose	Rats were examined 1 year after birth. **Leydig Cell:** Positive (for proliferative activity as indicated by presence of hyperplasia) There was an increase in the incidence of Leydig cell hyperplasia in 5-AzaC-treated rats (6/9 vs. 6/49 controls). Statistical analysis was not performed.	Carr et al. (1988)
F344 rats (young adults; 160-180 g)	10M (LD) 10M (MD) 100M (HD)	50M (age controls given saline i.p. 3 times/wk for 1 yr)	5-AzaC, purity not specified	0.025, 0.25, or 2.5 mg/kg bw (0.1, 1.0 or 10 µmol/kg bw) in saline, 3 times/wk i.p. [Regimens 2-4 in Tables I and II of the paper]	1 yr	Rats were killed at the end of the 1-year treatment period, at which time all LD and MD, but only 87/100 HD, rats were alive. **Leydig Cell:** Negative There was no significant increase in the incidence of Leydig cell hyperplasia in HD rats (11/87 vs. 6/49). Leydig cell hyperplasia was not detected in any of the LD or MD rats. IARC noted the "short duration of the experiment and the small numbers of animals in some groups."	Carr et al. (1988); IARC (1990)
Wistar rats (160-165 g)	6F (3-day treatment) 6F (4-day treatment)	6F It was not specified if controls received vehicle or were left untreated.	5-AzaC, purity not specified	5 µmol/100 g/day [12 mg/kg/day] i.p.	3 or 4 days	Twenty-four hours after the last injection of 5-AzaC, [2-^{14}C]thymidine or [^{3}H]thymidine was administered. Two hours later, the animals were killed and livers were removed. **Liver:** Positive (for proliferative activity as indicated by labeling, synthetic, and mitotic indices) Thymidine incorporation into liver DNA, the synthetic index, and the mitotic index were increased in 5-AzaC-treated rats (labeling index: 8700 ± 1360 dpm/µmol after 3 days, 18040 ± 1420 dpm/µmol after 4 days, vs. 1740 ± 255 dpm/µmol in controls; synthetic index: 73.3 ± 13.0 after 3 days, 100.5 ± 20.2 after 4 days, vs. 22.5 ± 4.9 in controls; mitotic index: 0.25 after 3 days, 0.18 after 4 days, vs. 0 in controls). There was no change in the level of thymidine-kinase or thymidylate-kinase in 5-AzaC-treated rats as compared to controls.	Čihák and Seifertová (1976)

Table 6-3. Cell Proliferation Induced by Azacitidine

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Transplacental Administration*							
pregnant F344 rats on day 21 of timed pregnancies	5F	50M (age controls given saline i.p. 3 times/wk for 1 yr)	5-AzaC, purity not specified	10 mg/kg bw (41 µmol/kg bw) i.p. in saline [Regimen 11 in Tables I and II of the paper]	single dose	Offspring (13 males, 9 females) were examined 1 year after birth. **Leydig Cell:** There was a slight increase in the incidence of Leydig cell hyperplasia in offspring of 5-AzaC-treated rats (3/22 vs. 6/49). Statistical analysis was not performed.	Carr et al. (1988)

Abbreviations: bw = body weight; F = females; HD = high dose; i.p. = intraperitoneally; LD = low dose; M = males; MD = mid dose

Table 6-4. Mammalian Carcinogenicity of Azacitidine in Combination with Other Treatments

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Benzo[*a*]pyrene (B[*a*]P) and 5-AzaC							
outbred Syrian golden hamsters (age not specified)	B[*a*]P + 5-AzaC (number and sex exposed not specified)	B[*a*]P alone (number and sex exposed not specified)	5-AzaC, purity not specified	5 mg/kg (20 µmol/kg) twice/wk i.p.	up to 70 wk	B[*a*]P (10%) was administered in sustained release implants (SRIs). SRIs were placed in the right lower lobe bronchus via tracheostomy. 5-AzaC-treatment of ½ of the hamsters was begun after B[*a*]P-treatment; ½ of the hamsters had no further treatment. Hamsters from both groups were killed at 3-wk intervals beginning 61 days after SRI placement. "The bronchial epithelium at the SRI site was examined histologically, as was the contralateral bronchial epithelium in the 5-AzaC-treated hamsters". **Lungs:** "The SPC [sequential progression of carcinogenesis] was slower in 5-AzaC-treated hamsters and the size of the cancers that developed was significantly less than in the controls". No data were presented.	Hammond and Benfield (1993)
inbred (syngeneic) Syrian golden hamsters (age not specified)	1) B[*a*]P + 5-AzaC (5-AzaC twice/wk throughout experiment) 2) B[*a*]P + 5-AzaC (5-AzaC twice/wk for 80 days, following B[*a*]P) 3) B[*a*]P + 5-AzaC (5-AzaC twice/wk beginning 80 days after B[*a*]P and continuing throughout rest of experiment) (number and sex exposed not specified)	B[*a*]P alone (number and sex exposed not specified)	5-AzaC, purity not specified	5 mg/kg (20 µmol/kg) twice/wk i.p.	up to 220 days	B[*a*]P (10%) was administered in sustained release implants (SRIs). SRIs were placed in the right lower lobe bronchus via tracheostomy. 5-AzaC-treatment was begun after B[a]P-treatment. Hamsters from each of the 4 treatment groups were killed at 80, 150, 180, or 220 days after SRI placement. "The bronchial epithelium at the SRI site and at similar areas in the contralateral bronchus was examined histologically." The following parameters were determined for each group of hamsters: the rate of sequential progression of carcinogenesis (SPC), the eventual incidence of cancers, the distribution of epidermoid and nonepidermoid cancers, the sizes of the cancers, and the degree of differentiation within the histologic pattern of the cancers. **Lungs:** There was no difference in any of the measured parameters between group 3 and the controls and between groups 1 and 2. In groups 1 and 2, however, most parameters were significantly different from group 3 and the control group: The rate of SPC was slower, the cancers were smaller and better differentiated, and there were fewer nonepidermoid cancers in the hamsters in groups 1 and 2 than in the hamsters in group 3 and the control group. No data were presented.	Hammond and Benfield (1993)

Table 6-4. Mammalian Carcinogenicity of Azacitidine in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
N-Nitrosodiethylamine (DEN) and 5-Azac							
F344 rats (age not specified)	10M per dose	12M (DEN alone)	5-AzaC, purity not specified	2.5 or 10 mg/kg bw (10 or 41 µmol/kg bw) in saline, twice/wk i.p.	9 mo	All rats (5-AzaC-treated and control) were administered DEN (50 mg/kg bw) i.p. 18 hours after partial hepatectomy. Although it appeared that DEN was given at the same time as 5-AzaC, it was not clear whether DEN was administered as a single dose or continuously over the 9-month 5-AzaC-treatment period. All rats were killed at 18 months. Statistical analysis of tumor incidence was not mentioned in the IARC review. **Liver:** Liver tumors (type not specified) were detected in rats treated with 5-AzaC + DEN (2/10 LD, 8/10 HD), but not in rats treated only with DEN. The number of controls used was not specified.	Carr et al. (1984; cited by IARC, 1990)
F344 rats (young adults, 160-180 g)	10M	50M (saline alone) No DEN controls were used.	5-AzaC, purity not specified	2.5 mg/kg bw (10 µmol/kg bw) in saline, 3 times/wk i.p. [Regimen 13 in Tables I and II of the paper]	1 yr	DEN (30 mg/kg [single dose]) was administered i.p. 18 hours after partial hepatectomy. It was not clear if 5-AzaC administration was begun immediately after DEN injection. **Testes:** Negative There was no significant increase in the incidence of testes tumors. **Other Tissues:** Positive The incidence of non-testis tumors was significantly increased in DEN + 5-AzaC-treated rats (5/8 [2 lung tumors, 3 skin tumors] vs. 0/49 in the controls; $p < 0.001$, Fisher's exact test).	Carr et al. (1988)

Table 6-4. Mammalian Carcinogenicity of Azacitidine in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Goitrogen Treatment and/or ^{131}I and 5-Aza							
28-day-old C3H × GPDX mice	9F (goitrogenic regime[1] + 5-AzaC) 9F (goitrogenic regime[1] + 5-aza-2-deoxycytidine [same dose regimen as 5-AzaC]) 11F (single i.p. injection of 5 µCi ^{131}I 7 days before start of goitrogenic regime[1])	9F (goitrogenic regime[1] alone)	5-AzaC, purity not specified	2.5 mg/kg (10 µmol/kg) i.p. in saline on days 5, 10, and 15 after start of goitrogenic regime	11 days	All mice were killed at 510 days of age. Thyroids were examined histologically. Statistical analysis of tumor incidence was not performed. **Thyroid:** Positive (for adenoma, carcinoma, and nodules) The incidence of thyroid tumors was increased in mice that received the goitrogen regime (GR) in combination with other treatments (GR + 5-AzaC: 3/8 [6 adenomas, 1 carcinoma, 2 nodules]; GR + 5-aza-2-deoxycytidine: 2/8 [5 adenomas, 2 nodules]; GR + ^{131}I: 10/11 [9 adenomas, 2 carcinomas, 11 nodules], vs. 0/8 controls). In one mouse treated with GR + 5-AzaC, a "very large carcinoma, occupying virtually the whole of one thyroid lobe" was detected. This tumor metastasized to the lungs and was positive for thyroglobulin on immunohistochemistry. The authors noted that in previous studies, they did not detect carcinoma in > 100 mice given goitrogen but no mutagen. It was noted that the demethylating agents (5-AzaC and 5-aza-2-deoxycytidine) were administered within the first two weeks of goitrogen treatment, when the follicular epithelial cells were at peak mitotic rate.	Thomas and Williams (1992)

[1]Goitrogenic regime = 0.2% aminotriazole, 0.5% sodium perchlorate, and 0.5% sucrose in drinking water from 28 days of age until 510 days of age
Abbreviations: bw = body weight; F = females; HD = high dose; i.p. = intraperitoneally; LD = low dose; M = males

Table 6-5. Cell Proliferation Induced by Azacitidine in Combination with Other Treatments

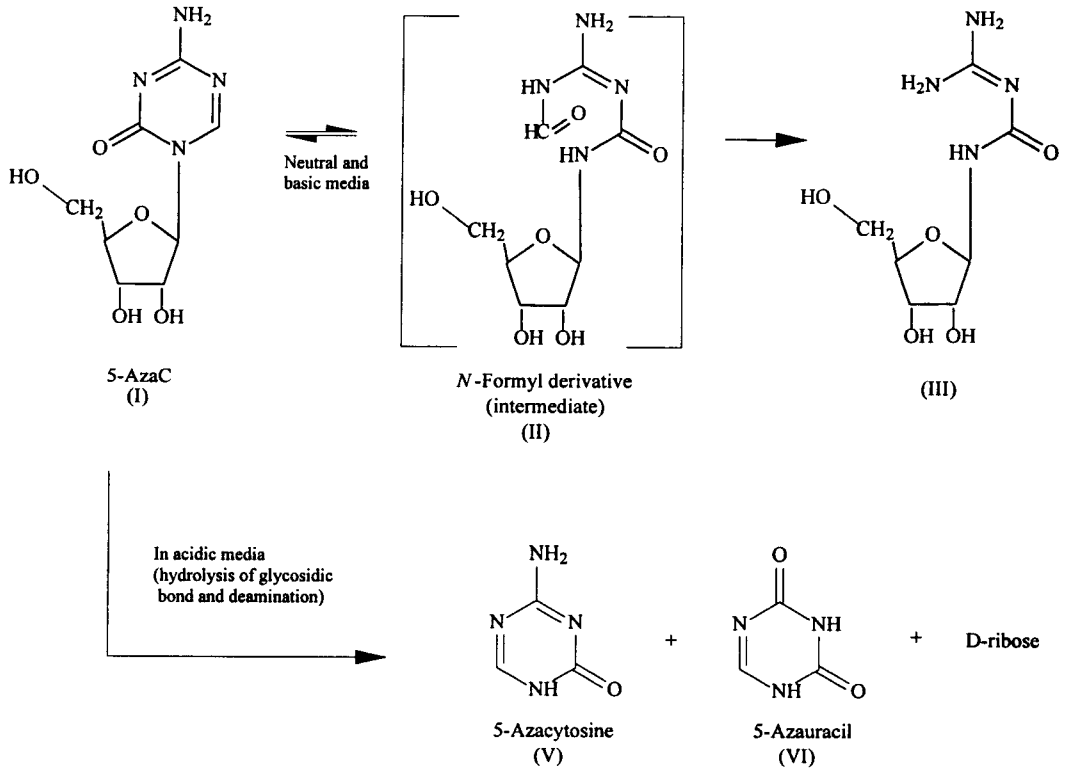
Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
21-day-old C3H x GPDX mice	1) 11F (single i.p. injection of 5 μCi ^{131}I at 21 days of age and 5-AzaC at 33, 38, and 43 days of age) 2) 11F (single i.p. injection of 5 μCi ^{131}I at 21 days of age and 5-aza-2-deoxycytidine at 33, 38, and 43 days of age) 3) 11F (single i.p. injection of 5 μCi ^{131}I at 21 days of age, goitrogen regime[1] staring at 28 days of age, and 5-aza-2-deoxycytidine at 33, 38, and 43 days of age) 4) 11F (single i.p. injection of 5 μCi ^{131}I at 21 days of age, goitrogen regime[1] staring at 28 days of age, and 5-AzaC at 33, 38, and 43 days of age)	none	5-AzaC, purity not specified	2.5 mg/kg (10 μmol/kg) i.p. in saline	11 days	All mice were killed at 510 days of age. Thyroids were examined histologically. Statistical analysis of tumor incidence was not performed. **Thyroid:** Thyroid tumors were detected in all groups of mice except those treated with ^{131}I + 5-AzaC (group 1: 0/9; group 2: 1/8 [adenoma]; group 3: 6/10 [4 adenomas, 2 carcinomas, 11 nodules]; group 4: 8/11 [14 adenomas, 1 carcinoma, 13 nodules]). It was noted that the demethylating agents (5-AzaC and 5-aza-2-deoxycytidine) were administered within the first two weeks of goitrogen treatment, when the follicular epithelial cells were at peak mitotic rate.	Thomas and Williams (1992)

Table 6-5. Cell Proliferation Induced by Azacitidine in Combination with Other Treatments (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
F344 rats (150-180 g)	1) B[*a*]P followed 4 h later with 5-AzaC 3) MNU followed 4 h later with 5-AzaC 5) DMH followed 12 h later with 5-AzaC 6) DMH followed 4 h later with 5-AzdC	2) B[*a*]P followed 4 h later with 0.9% NaCl 4) MNU followed 4 h later with 0.9% NaCl 7) DMH followed later with 0.9% saline; time not specified 8) 0.9% saline followed 4 or 12 h later with 5-AzaC 9) PH followed 18 h later with 5-AzaC 10) PH followed 48 h later with 5-AzaC	5-AzaC, purity not specified	10 mg 5-AzaC/kg [41 µmol/kg] i.p. 200 mg B[*a*]P/kg i.p. 60 mg MNU/kg i.p. 100 mg DMH/kg i.p. 2 mg 5-AzdC/kg i.p.	single dose	Rats in all groups were fed basal diet for 2 weeks after treatment. Rats were then fed a diet (for an additional 2 weeks) that contained 0.02% 2-acetylaminofluorene coupled with a necrogenic dose of CCl_4 to selectively stimulate initiated hepatocytes to form γ-glutamyltransferase (γ-GT) positive foci. The aim of the study was to administer carcinogens "at a dose which induced very few initiated hepatocytes", to administer 5-AzaC during the phase of DNA repair synthesis "to facilitate its incorporation into DNA and thereby create stretches of hypomethylated DNA", and to assay the initiated hepatocytes as foci of γ-GT positive hepatocytes. **Liver:** Positive (for proliferative activity with, as indicated by γ-GT positive foci) 5-AzaC, administered after B[*a*]P, MNU, or DMH, caused an increase in the incidence of initiated hepatocytes (number γ-GT positive foci/cm^2: 10 ± 1.2 [group 1], 3 ± 0.8 [group 2], 12 ± 0.3 [group 3], 5 ± 0.1 [group 4], 20 ± 2.1 [group 5], 9 ± 4.3 [group 6], 3 ± 1.2 [group 7], and none in groups 8, 9, and 10). Statistical analysis was not performed.	Denda et al. (1985)

Abbreviations: 5-AzdC = 5-azadeoxycytidine; B[*a*]P = benzo[*a*]pyrene; DMH = 1,2-dimethylhydrazine; F = females; i.p. = intraperitoneally; MNU = *N*-methyl-*N*-nitrosourea; PH = 2/3 partial hepatectomy

Figure 6-1. Hydrolysis Products of Azacitidine *In Vitro*



Source: Pithova et al. (1965; cited by von Hoff and Slavik, 1977)

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APPENDIX A

DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR AZACITIDINE

DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR AZACITIDINE (IARC Monograph in Vol. 50, 1990)

The searches described below were conducted between March and October 1996. An exhaustive search of all pertinent databases was not attempted, but the ones chosen were expected to provide citations for most of the relevant recently published literature. No attempt was made in the search strategy to find toxicity information for metabolites and other structural analogues.

Generally, if an IARC monograph or another authoritative review had been published, literature searches were generally restricted from the year before publication to the current year.

Older literature that needed to be examined was identified from the reviews and original articles as they were acquired. Current awareness was maintained by conducting weekly searches of Current Contents on Diskette Life Sciences 1200 [journal] edition.

In the searches, two variant spellings were used for the name—azacitidine (which is 5-azacytidine) and azacytidine. The term azacytidine is a component of several biologically active compounds, including deoxy-, dihydro-, and arabinosyl- analogues of azacitidine.

TOXLINE (on STN International): In the entire database (1965 to 06 March 1996), 761 records were indexed by the Chemical Abstracts Service Registry Number (CASRN) 320-67-2; 62 by the drug's generic name azacitidine; and 978 by azacytidine, which, as noted above, will bring in records related to structural analogues. A total of 1052 records were indexed by one or more of the terms. The set was reduced by combining it with all NLM Medical Subject Headings for metabolism (92 terms) and neoplasms (654 terms) and by combining it with the free text truncated terms (denoted by ?) "carcinogen? or mechanism? or toxicokinetic? or pharmacokinetic?" The results from these 3 combinations were 312, 430, and 38; and when the answer sets were combined, the total number of records was 574. The titles of the 574 records were examined in March to identify toxicity and metabolism publications and reexamined in July when the mechanism of action was researched. In consultation with Dr. Raymond W. Tennant, at least 32 publications were selected for acquisition.

CANCERLIT: The same strategy as described for TOXLINE was used in CANCERLIT. The resultant number of records was 424. Only 2 publications of interest appeared to be unique to this database.

EMIC/EMICBACK: Thirty-two records were indexed by the CASRN in EMIC and 167 in EMICBACK. Fifteen recent articles were selected for acquisition.

EMBASE: The same strategy as described for TOXLINE was used in EMBASE. The resultant number of records was 213. Two unique publications were of interest.

IRIS: In the entire database the numbers of records indexed by the CASRN, azacitidine, and azacytidine were 1090, 1304, and 1201, respectively. The total number of records indexed by one or more of the terms was 1625. The records were reduced to 1151 by combining with free text truncated terms in the statements "carcinogen? or mechanis? or toxicokinetic? or pharmacokinetic? or metaboli? or neoplas? or hyperplas? or metaplas? or foci or tumor? or tumour?". Of the 1151, 462 were published after 1988, and 458 did not include the controlled indexing vocabulary term "therapy." About 30 publications were selected for retrieval.

NTIS: Several older reports were indexed. One recent 1993 report on DNA-active developmental toxicants was considered but not selected because the title compound was 5-fluorouracil.

TOXLIT: The same strategy that was used in TOXLINE was used in TOXLIT. The resulting number of records was 122. Fourteen unique publications of interest were selected for acquisition.

In September 1996, the contractor performed searches for updating sections 1 and 2, which had been last updated in 1994 with regulatory information from print sources and REGMAT (May 1993 version). REGMAT had broad coverage of EPA regulations, but it is no longer available. Databases searched in 1996 included CSCHEM and CSCORP for U.S. suppliers (databases produced by Chem Sources); HSDB; the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System) and ISHOW (for physical-chemical properties); Chemical Abstracts Service's (CAS) File CHEMLIST for TSCA and SARA updates in 1996; and CAS's CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information.

In further attempts to identify pertinent FDA regulations and the current usage status (approved or investigational), another series of searches in September 1996 were performed in pharmaceuticals and other regulatory databases. The databases included the following:

- 21 CFR (via Internet access)
- Clinical Pharmacology (drug monographs available on the Internet from Gold Standard Multimedia, Inc.
- Derwent Drug File (DIALOG File 376 for nonsubscribers) (covers 1964-1982)
- Diogenes (DIALOG File 158) (covers 1976-1996; file includes FDA regulatory information from news stories and unpublished documents, including listings of approved products, documentation of approval process for specific products, recall, and regulatory action documentation)
- Drug Data Report (DIALOG File 452) (covers 1992-1996)

- Drug Information Fulltext (DIALOG File 229) (current, updated quarterly; includes information on at least 1000 commercially available drugs and 57 investigational injectable drugs)

- Federal Register (DIALOG File 136) (cover 1988-1996) (full text)

- Federal Register Abstracts (DIALOG File 136) (covers 1977-1993)

- International Pharmaceutical Abstracts (DIALOG File 74) (covers 1970-1996, all phases of drug development including laws and state regulations)

- NCI/PDQ. National Cancer Institute's menu-driven online database available from the National Library of Medicine and via the Internet. File contains state-of-the-art cancer treatment protocols and clinical trials. 1996.

- PHIND (Pharmaceutical and Healthcare Industry News Database, DIALOG File 129) (covers 1980-1996)

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange