NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for CHLOROZOTOCIN

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NTP Report on Carcinogens Listing for Chlorozotocin

Carcinogenicity

Chlorozotocin is *reasonably anticipated to be a human carcinogen* based on evidence from studies in experimental animals (reviewed in IARC, 1990), and because it is a member of a well defined, structurally-related class of substances listed in a previous Annual or Biennial Report on Carcinogens as either known to be human carcinogens, or reasonably anticipated to be human carcinogens (NTP, 1994).

When administered intraperitoneally to male and female rats, chlorozotocin induced an increase in the combined incidence of sarcoma and mesothelioma in the peritoneal cavity. When administered intravenously to male rats, chlorozotocin induced increases in the formation of malignant tumors of the nervous system, lungs, and forestomach.

There are no adequate data available to evaluate the carcinogenicity of chlorozotocin in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Chlorozotocin is an alkylating agent and is structurally related to other chloroethyl nitrosoureas, one of which, 1-(2-chloro)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU), is listed in the NTP Report on Carcinogens as a *known human carcinogen* and two of which, *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) are listed in the NTP Report on Carcinogens as *reasonably anticipated to be human carcinogens* (NTP, 1994). Chlorozotocin exhibits genetic activity in a wide variety of assays in bacteria and mammalian cells, inducing mutations in bacteria, yeast, insects, and cultured mammalian cells, and DNA damage in human, mouse, and Chinese hamster cells *in vitro,* and in bone-marrow cells in rats *in vivo.* Chlorozotocin exerts its adverse effects through the formation of mono- and bifunctional alkylating agents.

No data are available that would suggest that the mechanisms thought to account for tumor induction by chlorozotocin 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

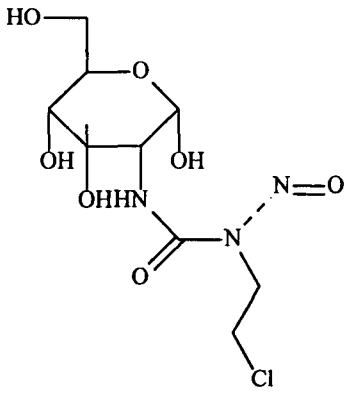
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to 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 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 judgment, 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

Chlorozotocin
[54749-90-5]



1.1 Chemical Identification

Chlorozotocin ($C_9H_{16}ClN_3O_7$), mol. wt. = 313.69) is also called:

1-(2-Chloroethyl)-1-nitroso-3-(D-glucos-2-yl)urea
2-[3-(2-Chloroethyl)-3-nitrosoureido]-D-glucopyranose
D-Glucopyranose, 2-[[[(2-Chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-
D-Glucose, 2-[[[(2-Chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-DCNU

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Ivory	Budavari (1996)
Physical State	Solid, Crystalline	Budavari (1996)
Melting Point, °C	147-148, decomposition with evolution of gas	Budavari (1996); (Burns, Heidel)
	141, decomposition	Budavari (1996); (Johnston)
Solubility:		
Water at 20 °C	Soluble in water	Budavari (1996)
Organic Solvents	not provided	Budavari (1996)

1.3 Identification of Structural Analogues and Metabolites

Several structural analogues and metabolites discussed in this report may be found in Figures 6-1 and 6-2.

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

Chlorozotocin (CZT; NSC 178248) is a cytostatic agent that is used in the investigational treatment of cancers of the stomach, large intestine, pancreas and lung; melanoma; and multiple myeloma. It has been given intravenously (i.v.) at doses of 100 to 225 mg/m^2 (IARC, 1990).

The oldest 5 records on CZT use were published in 1975 (Derwent Drug File, 1964-1982). Publications from 1975 through 1992 include Phase I clinical studies (International Pharmaceutical Abstracts, 1970-1996).

2.2 Production

CZT is reported to be produced in the United States, but no production data have been reported in appropriate online and print references consulted in 1996. No data on imports or exports of chlorozotocin were available. It is not sold commercially by U.S. suppliers (not listed in Chem Sources, 1996).

CZT can be synthesized by nitrosation of the urea derivative prepared from D-glucosamine and 2-chloroethyl isocyanate (IARC, 1990). Synthesis of CZT was reported in 1975 (Johnston et al., 1975; cited by IARC, 1990).

2.3 Environmental Exposure

Potential exposure of health professionals may occur during the preparation and administration of CZT. Potential occupational exposure may also occur for workers involved in the formulation and packaging of the pharmaceutical. The National Occupational Exposure Survey (1981-1983) indicated that 267 workers, including 223 women, were potentially exposed to chlorozotocin (NIOSH, 1984). This estimate was derived from observations of the use of the actual compound (100% of total observations). CZT is not a naturally occurring substance.

2.4 Regulations

No regulations pertaining to CZT were found by name or CAS Registry Number.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of CZT in humans.

4.0 MAMMALIAN CARCINOGENICITY

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

Summary: There is "sufficient evidence" for the carcinogenicity of CZT in experimental animals (IARC, 1990). The combined incidence of sarcoma and mesothelioma of the peritoneal cavity was significantly increased in male and female rats administered CZT once per week intraperitoneally (i.p.) for up to 800 days. The incidences of malignant tumors of the nervous system, lungs, and forestomach were slightly increased in male rats (females not evaluated) administered CZT intravenously (i.v.) every 6 weeks for 54 weeks. No mouse assays were found.

4.1 Intraperitoneal Injection

The combined incidence of sarcoma and mesothelioma (individual incidences not given) of the peritoneal cavity was significantly increased in male and female Sprague-Dawley rats administered CZT (0.4 or 2.0 mg/kg bw [1.3 or 6.4 μmol/kg bw]) once per week i.p. from age 100 days for up to 800 days (14/20 low-dose and 13/20 high-dose males vs. 0/20 controls; 10/20 low-dose and 16/20 high-dose females vs. 1/20 controls) (Habs et al., 1979).

4.2 Intravenous Injection

A slight increase in the incidence of malignant tumors (types not specified) of the nervous system (4% vs. 1% in the controls), lungs (5% vs. 0%), and forestomach (4% vs. 1%) were detected in male Wistar rats (age not specified; females not evaluated). CZT was administered (9.5, 19, or 38 mg/m^2 [30, 61, or 121 μmol/m^2]) by i.v. injection every 6 weeks for 54 weeks. Actual incidences were not given and the percent of tumors in each group was not broken down by dose. Also, no mention was made of statistical analysis (Eisenbrand and Habs, 1980; Eisenbrand et al., 1981; both cited by IARC, 1990; Zeller et al., 1982).

Table 4-1. Mammalian Carcinogenicity of Chlorozotocin

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Intraperitoneal Injection*							
100-day-old Sprague-Dawley rats	20M, 20F per dose	20M, 20F (vehicle alone)	CZT, purity not specified	0.4 or 2.0 mg/kg bw (1.3 or 6.4 µmol/kg bw), once/wk	up to 800 days	Median survival of CZT-treated rats was decreased (463 and 307 days for LD and HD males, respectively, vs. 724 days for male controls; 694 and 346 days for LD and HD females, respectively, vs. 750 days for female controls). Statistical analysis was performed using Fisher's exact test (IARC, 1990). Habs et al. (1979) stated that statistical analyses "were done using the U-test of Wilcoxon, Mann, and Whitney or the χ^2-test." **Peritoneal Cavity:** Positive (for sarcoma and mesothelioma at either dose) The combined incidence of sarcoma and mesothelioma (individual incidences not given) was increased in all groups of CZT-treated rats (14/20 LD [$p < 0.001$] and 13/20 HD [$p < 0.001$] males vs. 0/20 controls; 10/20 LD [$p = 0.002$] and 16/20 HD [$p < 0.001$] females vs. 1/20 controls). The mean induction time of i.p. tumors was significantly shorter in the HD than in the LD rats (336 vs. 472 days; $p < 0.05$).	Habs et al. (1979)
Rats - *Intravenous Injection*							
Wistar rats (age not specified)	30M per dose	120M (vehicle alone)	CZT, purity not specified	9.5, 19, or 38 mg/m^2 (30, 61, or 121 µmol/m^2) every 6 weeks for a total of 10 applications	54 wk	Rats were observed for life. Median survival time was as follows: 583, 590, and 474 days for LD, MD, and HD rats, respectively, vs. 621 days for controls. **Nervous System, Lungs, Forestomach:** Malignant tumors (types not specified) of the nervous system, lungs, and forestomach were detected in 4%, 5%, and 4%, respectively, of CZT-treated rats vs. 1, 0, and 1%, respectively, of controls. Actual incidences and percent of tumors in each dose group were not given. No mention was made of statistical analysis. In the sketchy review by Zeller et al. (1982), the treatment was reported to have induced 1 peripheral neurogenic sarcoma and 2 brain sarcomas among the 90 dosed animals, yet 38 mg/m^2 was stated to be "the lowest dose which proved to increase cancer risk in male Wistar rats (two-tailed $p < 0.005$)." The solvent control group (120 rats) exhibited 1 astrocytoma in the brain and 1 peripheral neurogenic sarcoma. IARC noted the poor survival of the CZT-treated rats and the limited reporting.	Zeller et al. (1982) (also cited by IARC, 1990) Eisenbrand and Habs (1980) and Eisenbrand et al. (1981); cited by IARC (1990)

5.0 GENOTOXICITY

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

Summary: CZT was found to be genotoxic in a wide variety of noneukaryotic, lower eukaryotic, and mammalian *in vitro* test systems. CZT was found to induce gene mutations in *Salmonella typhimurium* and Chinese hamster V79 cells; mitotic gene conversions in yeast; sex-linked recessive mutations in *Drosophila*; DNA damage in Chinese hamster V79 and mouse leukemia cells, cross-links in calf thymus DNA and human embryo cells; sister chromatid exchanges in mouse leukemia and rat brain tumor cells; inhibition of DNA synthesis in mouse bone marrow cells; and DNA strand breaks and interstrand cross-links in the bone marrow cells of treated rats. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*.

5.1 Noneukaryotic Systems

5.1.1 DNA Damage

Vadi and Reed (1983) reported that CZT exposure for 1 to 4 hours induced DNA damage, as measured by gel electrophoresis of nicked plasmid forms followed by scintillation counting of the bands; DNA alkylation, as measured by the binding of [^{14}C]CZT; and DNA interstrand cross-links, as measured following neutral sucrose denaturation, in pBR322 plasmid DNA [LED = 2857 μg/mL (9100 μM) for all three assays].

5.1.2 Gene Mutations

As reviewed by IARC (1990), two separate papers (Franza et al., 1980; Suling et al., 1983) found CZT to induce base-pair substitutions in *Salmonella typhimurium* [strains TA100 (LED =62 μg/plate; 0.2 μmol/plate) and TA1535 (doses not given in original)] but not frameshift mutations [strains TA98 and TA1538, HID = 124 μg/plate (0.4 μmol/plate)] in both the presence and absence of metabolic activation. Zimmer and Bhuyan (1976; cited by IARC, 1990) reported that CZT induced reverse mutations in *S. typhimurium his*G46 only in the absence of metabolic activation [LED = 200 mg/plate (0.63 μmol/plate)].

5.2 Lower Eukaryotic Systems

Siebert and Eisenbrand (1977; cited by IARC, 1990) reported that chlorozotocin induced mitotic gene conversions in the fungus *Saccharomyces cerevisiae* in the absence of metabolic activation [LED = 314 μg/mL (1000 μM)]. Kortselius et al. (1978; cited by IARC, 1990) found that, in the fruit fly, *D. melanogaster*, chlorozotocin induced sex-linked recessive mutations in the absence of metabolic activation [LED = 31.4 μg/mL (100 μM)].

5.3 Mammalian Systems *In Vitro*

5.3.1 DNA Damage

Erickson et al. (1980; cited by IARC, 1990) stated that CZT at 50 to 200 μM for 2 hours, followed by 300 rad X-rays, was found to cause DNA interstrand cross-links in SV40 transformed human embryo cells [LED = 15.7 μg/mL (50 μM)]. Alexander et al. (1986; cited by IARC, 1990) also reported that 5 μM CZT for 24 hours induced DNA cross-links in calf thymus DNA in the absence of metabolic activation, as measured by fluorescence with ethidium bromide following denaturation and renaturation [LED=1570 mg/mL (5M)]. Alexander et al. (1986; cited by IARC, 1990) also used alkaline elution to determine that CZT tested at 5.0 to 50 μM for 2

activation [LED=15.7 mg/mL (50 μM)]. Erickson et al. (1988; cited by IARC, 1990) stated that CZT (dose range not provided) induced DNA strand breaks in V79 Chinese hamster lung cells [LED = 4 μg/mL (13 μM)].

Tofilon et al. (1983; cited by IARC, 1990) reported that sister chromatid exchanges (SCE) were induced by CZT in rat brain tumor 9L cells [LED = 0.3 μg/mL (1 μM)]. Siddiqui et al. (1988; cited by IARC, 1990) also reported that SCEs were induced by CZT in mouse leukemia L1210 cells [LED = 0.1 μg/mL (0.3 μM)].

Ali-Osman et al. (1985) reported that CZT at 10 to 100 μM for 2 hours inhibited DNA synthesis (by 53% at the highest dose), but not RNA (3%) or protein (2%) synthesis, in DBA/2 mouse bone marrow cells. Reductions in synthesis were measured by incorporation of [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]leucine, respectively.

5.3.2 Gene Mutations

Bradley et al. (1980; cited by IARC, 1990), found that CZT exposure at 20 μM for 2 hours in the absence of metabolic activation, followed by a 1- to 9-day expression period, induced mutations at the *hprt* locus in Chinese hamster lung V79 cells.

5.4 Mammalian Systems *In Vivo*

Bedford and Eisenbrand (1984; cited by IARC, 1990) reported that female Wistar rats administered CZT at 100 μmol/kg i.p., followed by sacrifices at 6 to 48 hours post injection, exhibited both DNA strand breaks and interstrand DNA cross-links (as measured by alkaline elution) in their bone marrow cells. The level of DNA strand breaks peaked at 36 hours while DNA cross-links peaked earlier at 6 hours.

Table 5-1. Summary of Chlorozotocin 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							
pBR322 Plasmid DNA	DNA damage (gel electrophoresis of nicked plasmid forms followed by scintillation counting)	-	n.p.	9.1 and 45.5 mM (9100 to 45,500 mM) CZT plus [^{3}H]pBR322 for 1 to 4 h	positive	LED = 2857 μg/mL (9100 μM)	Vadi & Reed (1983)[a]
pBR322 Plasmid DNA	DNA alkylation (binding of [^{14}C]CZT)	-	n.p.	9.1 to 27.3 mM (9100 to 27,300 mM) [^{14}C]CZT for 2 h	positive	LED = 2857 μg/mL (9100 μM)	Vadi & Reed (1983)[a]
pBR322 Plasmid DNA	DNA interstrand cross-links (neutral sucrose denaturation)	-	n.p.	9.1 mM (9100 mM) CZT for 1 to 3 h	positive	LED = 2857 μg/mL (9100 μM)	Vadi & Reed (1983)[a]
5.1.2 Gene Mutations							
Salmonella typhimurium strains TA100, TA98, TA1535, TA1537, and TA1538	*his* gene mutations	+/-	n.p.	n.g.	positive/ positive	Positive for base pair substitutions in strains TA100 [LED = 62 μg/plate (0.2 μmol/plate)] and TA1535 [doses not give in original] but not frameshift mutations in strains TA98 and TA1538 [HID =124 μg/mL (0.4 μmol/plate)	Franza et al. (1980), and Suling et al. (1983) cited by IARC (1990)
S. typhimurium hisG46	*his* gene mutations	-	n.p.	n.g.	positive	LED=200 μg/plate (0.63 μmol/plate)	Zimmer and Bhuyan (1976)[a]
5.2 Lower Eukaryotic Systems							
Saccharomyces cerevisiae	mitotic gene conversions	-	n.p.	n.g.	positive	LED = 314 μg/mL (1000 μM)	Siebert & Eisenbrand (1977) cited by IARC (1990)
Drosophila melanogaster	sex-linked recessive mutations	-	n.p.	n.g.	positive	LED = 31.4 μg/mL (100 μM)	Kortselius et al. (1978) cited by IARC (1990)

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

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.3 Mammalian Systems *In Vitro*							
5.3.1 DNA Damage							
human embryo cell lines IMR-90 and VA-13	DNA cross-links (alkaline elution plus x-rays)	-	n.p.	50 to 200 μM for 2 h followed by 300 rad x-rays	positive in VA-13 cells only	The SV40 transformed VA-13 cells exhibited a dose dependent increase in crosslinks whereas the normal IMR-90 cells did not. LED = 15.7 μg/mL (50 μM).	Erickson et al. (1980) cited by IARC (1990)
calf thymus DNA	DNA cross-links (fluorescence with ethidium bromide following denaturation and renaturation)	-	n.p.	1570 μg/mL (5000 μM) for 24 h	positive	LED = 1570 μg/mL (5000 μM)	Alexander et al. (1986) cited by IARC (1990)
mouse leukemia L1210 cells	DNA damage (alkaline elution)	-	n.p.	5.0 to 50 μM for 2 h	positive	LED = 15.7 μg/mL (50 μM)	Alexander et al. (1986) cited by IARC (1990)
Chinese hamster lung V79 cells	DNA damage (alkaline elution)	-	n.p.	n.g.	positive	LED = 4 μg/mL (13 μM)	Erickson et al. (1988) cited by IARC (1990)
9L rat brain tumor cells	sister chromatid exchanges (SCE)	-	n.p.	n.g.	positive	LED = 0.3 μg/mL (1 μM)	Tofilon et al. (1983) cited by IARC (1990)
mouse leukemia L1210 cells	sister chromatid exchanges	-	n.p.	n.g.	positive	LED = 0.1 μg/mL (0.3 μM)	Siddiqui et al. (1988) cited by IARC (1990)
DBA/2 mouse bone marrow cells	inhibition of DNA/RNA/Protein synthesis (incorporation of [^{3}H]thymidine/[^{3}H]uridine/ [^{3}H]leucine)	-	n.p.	10 to 100 μM for 2 h followed by 4 h with labeled precursors.	positive for DNA only	Inhibited DNA synthesis by 53%, RNA synthesis by 3%, and protein synthesis by 2% at the top dose.	Ali-Osman et al. (1985)
5.3.2 Gene Mutations							
Chinese hamster lung V79 cells	*hprt* gene mutations	-	n.p.	20 μM for 2 h followed by expression times from 1 to 9 days	positive	LED = 6.28 μg/mL (20 μM) starting at day 3 and plateauing from day 5 through 9.	Bradley et al. (1980) cited by IARC (1990)

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

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.4 Mammalian Systems *In Vivo*							
female Wistar rats	DNA damage in bone marrow cells (alkaline elution)	NA	n.p.	100 μmol/kg i.p. followed by sacrifices at 6 to 48 h	positive	LED = 31.4 mg/kg (100 μmol/kg) with peak at 36 h	Bedford & Eisenbrand (1984) cited by IARC (1990)
female Wistar rats	DNA interstrand crosslinks in bone marrow cells (alkaline elution)	NA	n.p.	100 μmol/kg i.p. followed by sacrifices at 6 to 48 h	positive	LED = 31.4 mg/kg (100 μmol/kg) with peak at 6 h.	Bedford & Eisenbrand (1984) cited by IARC (1990)

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; n.p. = purity not provided; n.g. = doses not given; NA = not applicable. bw = body weight; LD = low dose; MD = mid dose; HD = high dose

Figure 5-1. Genetic Activity Profile of Chlorozotocin

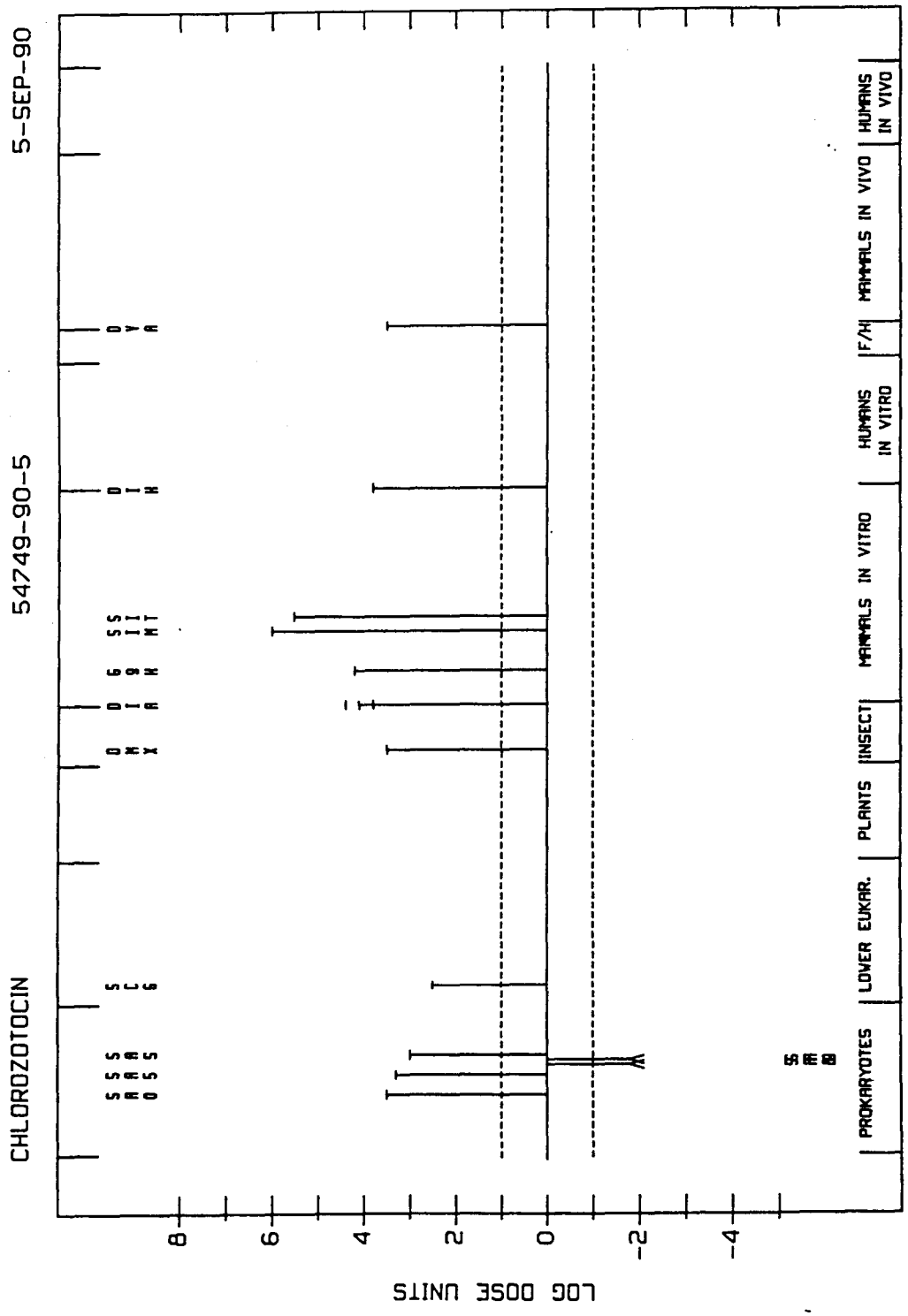
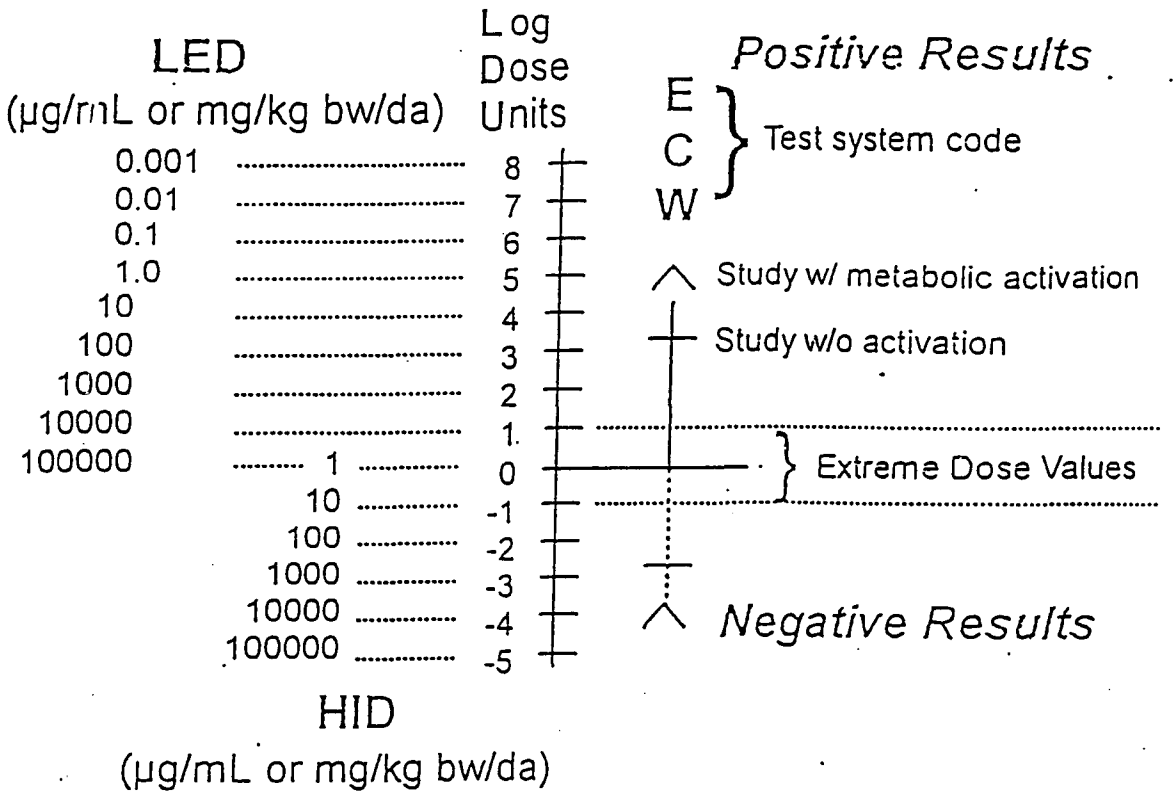


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



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: The metabolism of nitrosoureas has been reviewed by Wang et al. (1981) and Lemoine et al. (1991). No other data were found for the absorption, distribution, metabolism, and excretion of CZT. CZT belongs to a group of alkylating antineoplastic agents that decompose to 2-choroethylnitrosoureas (CENUs). Protein binding enhances the decomposition of CENUs. Decomposition of CENUs following protein binding is probably the main mechanism by which this group of compounds elicit their cytotoxic effects.

The primary mechanism of action of CENUs is most likely via alkylation. Many volatile products have been identified by gas chromatography as a result of chemical degradation of CENUs. 2-Chloroethanol (ethylene chlorohydrin) constitutes one of the major volatile products that is thought to result from hydroxylation of the alkylating carbonium ion. Oxidative dechlorination is the major inactivation metabolic pathway of CENUs. Cytosol is probably necessary in this metabolic pathway. Cytochrome P-450 is involved in one of the several steps in oxidative dechlorination of CENUs as shown by *in vitro* and *in vivo* experiments. Although considered less important than oxidative dechlorination, denitrosation is another well established means of metabolic deactivation of CENUs, representing 5 to 14% of the metabolic fate of these drugs. Cytosol has been implicated in the denitrosation of CENUs and is catalyzed by a glutathione-dependent enzyme.

6.1.1 Chemical Decomposition of 2-Chloroethylnitrosoureas (CENUs)

CZT belongs to a group of alkylating antineoplastic agents that decompose to 2-choroethylnitrosourea. Under physiological conditions, nitrosoureas spontaneously decompose (Colvin et al., 1976; Montgomery et al., 1975; both cited by Wang et al., 1981) with half-lives ranging from a few minutes for 1-(2-chloroethyl)-1-nitrosourea (CNU) to 2 h for 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU; see Section 6.4) in phosphate buffered saline at pH 7.4 (Schein, 1978; cited by Wang et al., 1981; Lemoine et al., 1991). Protein binding enhances the decomposition of CENUs (Gordon et al., 1989; cited by Lemoine et al., 1991). Lemoine et al. (1991) stated that decomposition of CENUs following protein binding "is probably the main mechanism by which this group of compounds elicit their cytotoxic effects."

A series of alkylating moieties is formed during the degradation process, of which the highly reactive carbonium ion and alkyldiazo hydroxide are considered the most important due to their ability to alkylate the macromolecules within the tumor cell. Amino, carboxyl, sulfhydryl, and phosphate groups are the principal alkylation targets (Wang et al., 1981; Lemoine et al., 1991).

Organic isocyanates are produced from spontaneous decomposition of nitrosoureas and can carbamoylate intracellular proteins, depending on the size of the CENU in question (Schmall et al., 1973, and Wheeler et al., 1975; cited by Wang et al., 1981; Lemoine et al., 1991). CZT has negligible carbamoylating activity while retaining antitumor activity, suggesting that the primary mechanism of action of CENUs is via alkylation (Wang et al., 1981). Figures 6-1, 6-2, and 6-3 depict chemical decomposition of CENUs leading to alkylating and carbamoylating products and their subsequent glutathione adducts.

Many volatile products have been identified by gas chromatography as a result of chemical degradation of CENUs (Reed et al., 1975, and Taylor et al., 1979; cited by Lemoine et al., 1991).

2-Chloroethanol (ethylene chlorohydrin) constitutes one of the major volatile products that is thought to result from hydroxylation of the alkylating carbonium ion (Colvin et al., 1974, and Reed and May, 1975; cited by Lemoine et al., 1991). Studies conducted by Bass (personal communication; cited by Lemoine et al., 1991) have shown that in rats administered [^{14}C]2-chloroethanol, very little of the radio-labeled dose was expired in air as intact compound, with the majority of the dose (55%) detected in urine as three metabolites. Eighteen percent was expired as [^{14}C]CO_2, indicating metabolism to 1-C units. When incubated with human plasma protein *in vitro*, [^{14}C]2-chloroethyl was found to bind irreversibly to protein as a function of time at 37°C for up to 6 h. "However, it should be noted that CENUs are not highly protein bound (~25 to 35%)" (Lemoine et al., 1991).

6.1.2 Metabolism

Oxidative dechlorination is the major inactivation metabolic pathway of CENUs. Indicating that cytosol is necessary in this metabolic pathway, *in vitro* studies have shown that oxidative dechlorination of CENUs is considerably slower in pure microsomal preparations than in a crude S9 fraction (Lemoine et al., 1991). Cytochrome P-450 is involved in one of the several steps in oxidative dechlorination of CENUs as shown by *in vitro* and *in vivo* experiments (Nishigaki et al., 1985a, b; cited by Lemoine et al., 1991). Figure 6-1 depicts metabolic degradation of CENUs and Figure 6-4 depicts oxidative dechlorination of nitrosoureas.

Although considered less important than oxidative dechlorination, denitrosation is another well established means of metabolic deactivation of CENUs, representing 5 to 14% of the metabolic fate of these drugs (Weinkim and Lin, 1979; cited by Lemoine et al., 1991). Figures 6-1 and 6-4 depict denitrosation as a deactivation pathway of CENUs. Under aerobic or anaerobic conditions, this reaction can occur via several enzymes, including cytochrome P-450 and NADPH cytochrome P-450 reductase (Potter and Reed, 1982; cited by Lemoine et al., 1991). In the presence of mouse hepatic microsomes and NADPH, denitrosation occurs rapidly; however, this was not the case in similar incubations including NADH or NADP (Hill et al., 1975; cited by Lemoine et al., 1991).

Cytosol has been implicated in the denitrosation of CENUs (Smith et al., 1989; cited by Lemoine et al., 1991) and is catalyzed by a glutathione-dependent enzyme. "Glutathione adducts formed via glutathione transferase are invariably the main means of detoxification of reactive electrophilic intermediates such as the alkylating intermediates of the 2-chloroethylureas...." (Vermeulen, 1989; Vermeulen et al., 1989, 1990; all cited by Lemoine et al., 1991). See Section 6.3 for mechanistic implications.

6.2 Pharmacokinetics

The pharmacokinetics of CZT in humans have been reviewed by IARC (1990) and are discussed below.

In humans, i.v. administered CZT at 120 mg/m^2 (382 μmol/m^2), three successive exponential phases ($t_{1/2}$: 3-4.5 min, 6-12 min, and 18-30 min) were observed for the clearance of the *N*-nitroso group from circulating blood. Following administration of either [glucose-^{14}C]CZT or [ethyl-^{14}C]CZT, 82-84% of the blood-borne radioactivity was bound to protein after 24 h. Subsequently (7 days after administration), 2% of the peak radiolabel was detected in blood. Within 48 h, 58% of the radioactivity from [glucose-^{14}C]CZT and 50% of that from [ethyl-^{14}C]CZT was excreted in urine. Only 5-8% of the radiolabel was excreted as parent compound (Hoth et al., 1978; cited by IARC, 1990).

6.3 Modes of Action

CZT alkylates DNA and protein, causing DNA interstrand cross-links. This activity results in a broad spectrum of genetic damage (see section 5.0), including DNA damage and/or gene mutations in noneukaryotes, lower eukaryotes *in vitro* and *in vivo* mammalian systems. The *in vitro* effects occur in the absence of metabolic activation.

No information was found that would suggest that the biological activity and metabolic pathways of thiotepa in humans would differ from those in rodents sufficiently to suggest a different mechanism of carcinogenesis.

6.4 Structure-Activity Relationships

Summary: CZT is structurally related to three other chloroethylnitrosourea alkylating agents that have been found to be either carcinogenic to humans—1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; methyl-CCNU—or "probably carcinogenic to humans"—bis(chloroethyl)nitrosourea [BCNU] and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea [CCNU] (IARC, 1990). Methyl-CCNU, CCNU, and BCNU are directly acting, bifunctional alkylating agents. CZT is less closely related structurally to streptozotocin, a nitrosomethylurea.

6.4.1 Carcinogenicity of Structurally Related Compounds

6.4.1.1 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; Methyl-CCNU

Adjuvant treatment with methyl-CCNU was evaluated in 3633 patients with gastrointestinal cancer treated in nine randomized trials. Fourteen cases of acute nonlymphocytic leukemia occurred (ANLL; relative risk, 12.4; 95% confidence interval, 1.7-250) among 2067 patients treated with methyl-CCNU. Only 1 case was observed among 1566 patients treated with other therapies. At six years, cumulative (actuarial) risk was 4% and was not affected by concomitant immunotherapy or radiotherapy (Boice et al., 1986; cited by IARC, 1987a). A strong dose-response relationship (adjusted for survival time), giving a relative risk of almost 40-fold among patients who had received the highest dose, was observed by Boice et al. (1983; cited by IARC, 1987a).

In male rats, total tumor incidence was reported to be increased 1.5- to 2.0-fold over that in controls at 18 months following 3 i.p. injections/wk for 6 mo with methyl-CCNU. Similar exposure of mice induced a slight increase in tumor incidence (Weisburger, 1977; cited by IARC, 1987a). In rats, i.v. administration of methyl-CCNU induced lung tumors (Habs and Schmahl, 1984; cited by IARC, 1987a).

6.4.1.2 Bis(chloroethyl)nitrosourea (BCNU)

Limited evidence exists for carcinogenicity of BCNU to humans (IARC, 1987b). Within the first 2 years of treatment, ANLL occurred in 2 of 1628 patients treated with BCNU (0.08 expected) in 7 randomized trials of treatment of brain tumors. No such case was observed among 1028 patients not receiving BCNU treatment (Greene et al., 1985; cited by IARC, 1987b).

In rats, BCNU produced malignant tumors of the lung and increased risk of neurogenic tumors after repeated i.p or i.v. administration. Tumors in the peritoneal cavity were also observed in rats following i.p. administration of BCNU (IARC, 1981; Habs and Schmahl, 1984; Eisenbrand, 1984; cited by IARC, 1987b). In mice, BCNU caused an early appearance of skin tumors when tested by skin application together with UV radiation (IARC, 1981).

6.4.1.3 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)

No epidemiological studies of CCNU administered as a single agent were available for review by IARC (1987a). IARC has not reviewed the carcinogenicity of CCNU since 1987.

Following i.p. or i.v. injection of CCNU, lung tumors were produced in rats (IARC, 1981; Habs and Schmahl, 1984; cited by IARC, 1987a). CCNU induced a slight increase in the incidence of lymphomas in mice administered the compound i.p.; tests in rats could not be evaluated following oral administration (IARC, 1981). No skin tumors were observed in mice following skin application; duration of the experiment was deemed inadequate (Zackheim and Smuckler, 1980; cited by IARC, 1987a).

6.4.1.4 Streptozotocin (2-Deoxy-D-glucose derivative of 1-methyl-1-nitrosourea)

Streptozotocin is the 2-deoxy-D-glucose derivative of the nitrosamide carcinogen 1-methyl-1-nitrosourea. In rats, streptozotocin induced renal, liver, and pancreatic tumors (Arison and Fendall, 1967, Rakieten et al., 1968, Rakieten et al., 1971; Rakieten and Gordon, 1975; all cited by Vesely and Levey, 1978). It has also been shown to induce the activity of guanylate cyclase in rat and human tissues. See section 6.3 for relevance. Veseley and Levey (1978) found that streptozotocin analogues that have the nitroso group activate guanylate cyclase, and predicted that CZT would be carcinogenic when administered to animals.

6.4.2 Genotoxicity of Structurally Related Compounds

Methyl-CCNU, CCNU, and BCNU are directly acting, bifunctional alkylating agents (IARC, 1987a,b).

6.4.2.1 BCNU

In vitro experiments with human cells showed that BCNU induced DNA damage. In mice, BCNU induced chromosomal aberrations, micronuclei, and SCE. In rodent cells *in vitro,* BCNU induced aneuploidy, SCE, chromosomal aberrations, mutation, and DNA damage. In bacteria, BCNU also caused DNA damage and was mutagenic. In *Drosophila*, it induced sex-linked recessive lethal mutations and gene conversions in yeast (IARC, 1987b).

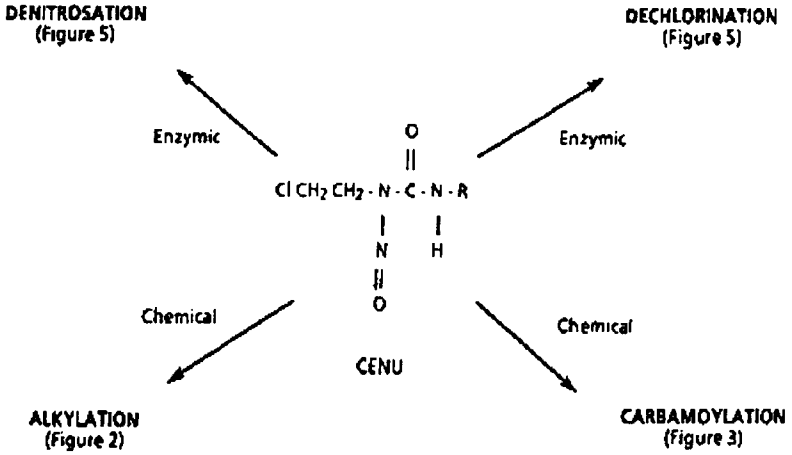
6.4.2.2 CCNU

In a single study of peripheral blood lymphocytes of patients treated with CCNU, an increased frequency of SCE was observed. In rats and mice, CCNU induced DNA damage and dominant lethal mutations in rats. In human and rodent cells *in vitro,* it induced DNA damage and SCE and mutation in cultured Chinese hamster cells. CCNU also induced DNA damage and mutation in bacteria (IARC, 1987a).

6.5 Cell Proliferation

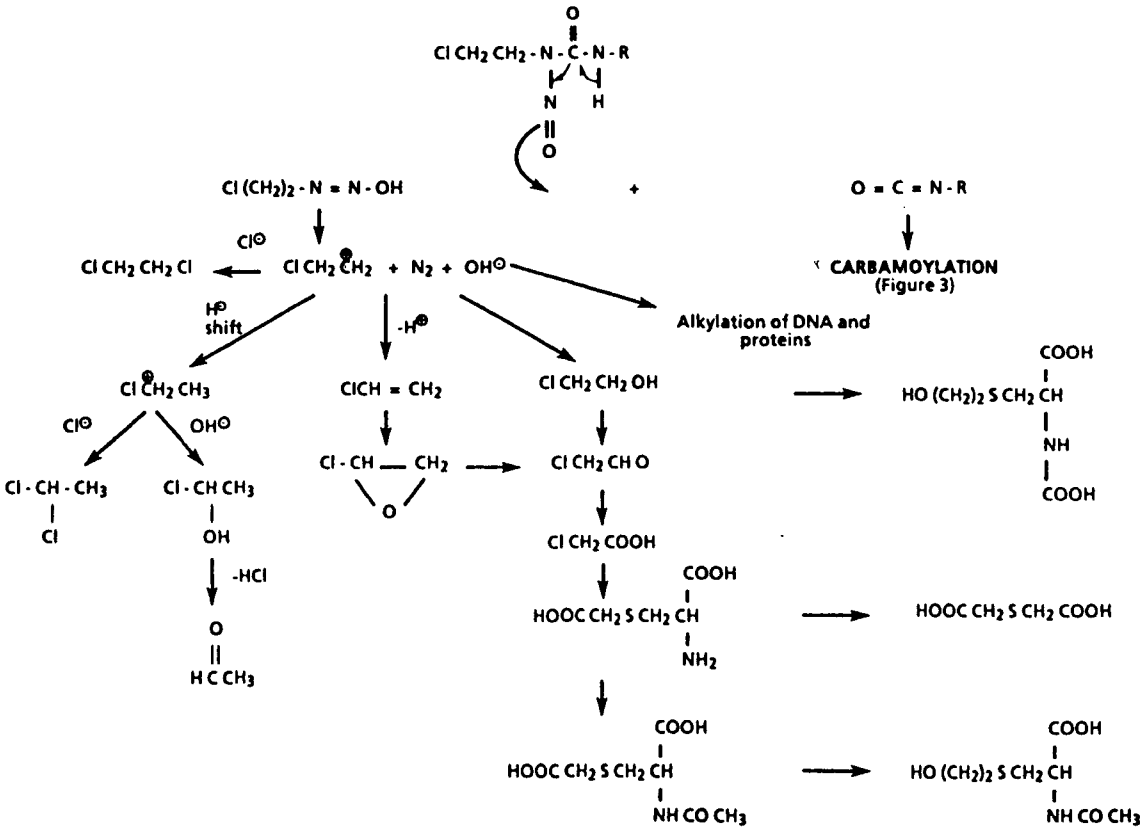
No studies were found that reported that chlorozotocin induced cell proliferation in experimental animals or in humans.

Figure 6-1. Chemical and Metabolic Degradation of Nitrosoureas



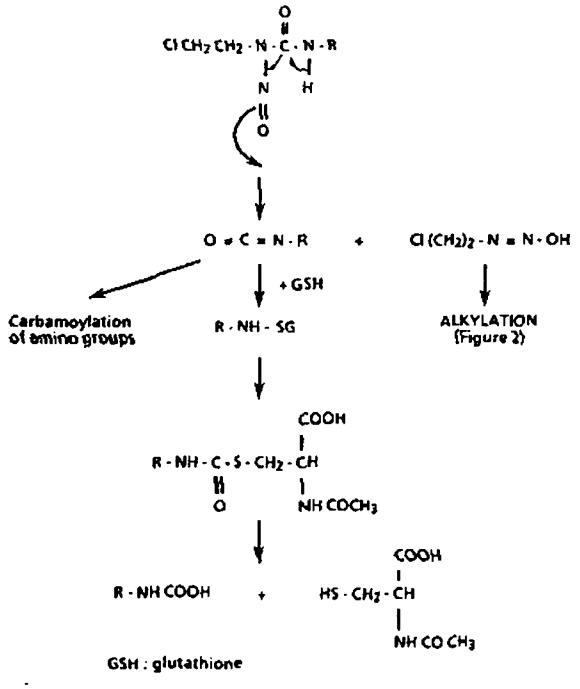
Source: Lemoine et al. (1991)

Figure 6-2. Chemical Degradation of Nitrosoureas Leading to Alkylating Products
Source: Lemoine et al. (1991)



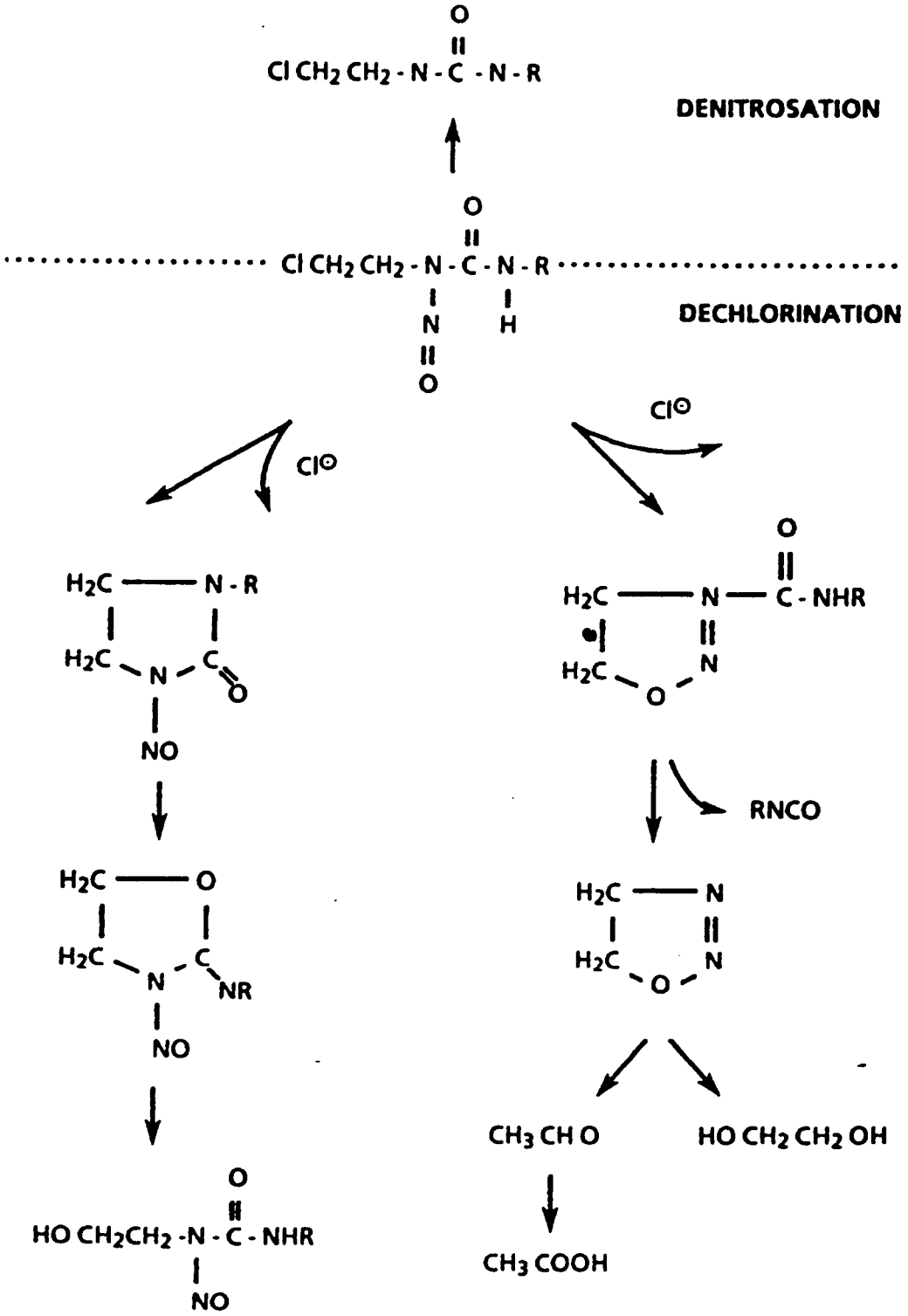
The chemical degradation of nitrosoureas leading to alkylating products, as described by Colvin *et al.* (1976), and some of the subsequent glutathione adducts.

Figure 6-3. Chemical Degradation of Nitrosoureas Leading to Carbamoylating Products and Their Subsequent Glutathione Adducts



Source: Lemoine et al. (1991)

Figure 6-4. The Metabolic Inactivation of Nitrosoureas



Source: Lemoine et al. (1991)

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR CHLOROZOTOCIN

DESCRIPTION OF ONLINE SEARCHES FOR CHLOROZOTOCIN (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 [journals] edition.

TOXLINE (on STN International): In the entire database (1965 to 06 March 1996), 201 records were indexed by chlorozotocin or the presumed misspellings chlorozoticin, chlorozotocine, or chlorozotozin. Only 9 represented publications after 1989. Among the remaining 192 records, 35 publications were selected for acquisition. When the Chemical Abstracts Service Registry Number (CASRN) was used, another 12 records were identified.

CANCERLIT: The same strategy described below under TOXLIT was used. The resultant number of records was 23. Three records unique to this database were selected for acquisition.

EMIC/EMICBACK: One record was indexed by the CASRN in EMIC and 67 in EMICBACK.

EMBASE: The same strategy as described below for TOXLIT was used in EMBASE. The resultant number of records was 79. Ten unique publications were selected for acquisition.

IRIS: No profile was found in this EPA risk assessment database.

MEDLINE: In the entire database the numbers of records indexed by chlorozotocin, chlorozoticin, or the CASRN were 199, 1, and 119, respectively. The total number of records indexed by one or more of the terms was 200. Of the 200, only 22 were published after 1988. All of the publications of interest had already been identified in TOXLINE or another database.

TOXLIT: A total of 203 records was indexed by the CASRN (183), chlorozotocin (135), or the misspelling chlorozoticin (1). Only 21 were published after 1988. Combination with truncated (use of ?) free text terms in the statement "carcinogen? or mechanis? or toxicokinetic? or pharmacokinetic? or metaboli? or neoplas? or hyperplas? or metaplas? or foci? or tumor? or tumour?" reduced the 21 records to a set of 13. Five 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 669) (covers 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)
- Unlisted Drugs (DIALOG File 140) (covers 1984-July 1994). In 1987, the manufacturer was listed as Southern Research Institute.

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