NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT FOR THIOTEPA

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NTP Report on Carcinogens Listing for Thiotepa

Carcinogenicity

Thiotepa is *known to be a human carcinogen* based on studies in humans which indicates a causal relationship between exposure to thiotepa and human cancer (reviewed in IARC, 1990).

Exposure to thiotepa is specifically associated with leukemia in humans. Adamson and Seiber (1981) summarized nine case reports from 1970 to 1978 of secondary development of nonlymphocytic leukemia occurring in cancer patients with primary cancers at other sites and who had received thiotepa as the only therapeutic agent. Additional evidence is found in a case-control study examining the development of leukemia as a secondary cancer in cancer patients undergoing chemotherapy compared to surgery alone. Patients undergoing chemotherapy were significantly more likely to develop secondary leukemia than those undergoing surgery alone, and in some of these patients, thiotepa was the only chemotherapeutic agent given (reviewed in IARC, 1990).

Thiotepa is carcinogenic at multiple sites in both sexes of mice and rats. In mice, thiotepa administered by intraperitoneal (i.p.) injection induced lymphoma or lymphocytic leukemia in both sexes and squamous-cell carcinoma of the skin and associated glands of males. In rats, i.p. injection of thiotepa induced squamous-cell carcinoma of the skin or ear canal in both males and females and neoplasms of the hematopoietic system in males (NCI, 1978). Other rodent studies using i.p. or intravenous (i.v.) routes of exposure found thiotepa to be carcinogenic as well. The incidence of lung tumors in both male and female mice was significantly increased by i.p. injections of thiotepa, and rats treated with thiotepa by i.v. injections developed benign and malignant tumors at multiple sites (reviewed in IARC, 1990).

Other Information relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Thiotepa and its major metabolite, tris(aziridinyl)phosphine oxide (also called TEPA and triethylenephosphoramide [generic name in *The Merck Index*]) are direct alkylating agents with potent genotoxic activity in a wide variety of prokaryotic, lower eukaryotic, and mammalian *in vitro* and *in vivo* test systems. Its ability to induce DNA damage, mutations, micronuclei, and/or chromosomal aberrations in somatic and germ cells sampled from treated rodents, rabbits, and primates, and chromosomal aberrations in peripheral blood lymphocytes sampled from treated humans (reviewed in IARC, 1990) are consistent with thiotepa being a genotoxic carcinogen.

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

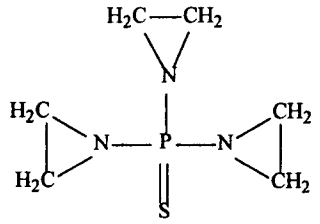
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to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

Thiotepa
[52-24-4]



1.1 Chemical Identification

Thiotepa ($C_6H_{12}N_3PS$, mol. wt. = 147.43) is also called:

Aziridine, 1,1',1"-phosphinothioylidynetris- (9CI)
Phosphine sulfide, tris(1-aziridinyl) (8CI)
Aziridine, 1,1,1"-phosphinothioylidynetris-
CBC 806495
Girostan
Ledertepa
NCI-C01649
NSC 6396
Oncotepa
Oncothio-Tepa
Oncotiotepa
1,1',1"-Phosphinothinoylidynetris(aziridine)
1,1',1"-Phosphinothioylidenetrisaziridine
1,1',1"-Phosphinothioylidynetrisaziridine
Phosphoric tri(ethyleneamide)
Phosphorothioic acid triethylenetriamide
Phosphorothioic triamide, *N,N',N"*-tri-1,2-ethanediyl-
Phosphorothioic triamide, *N,N',N"*-triethylene-
Tiofosfamid
Tiofosyl
Tiofozil
Phosphorothioic triamide, *N,N',N"*-triethylene
Phosphorothionic triethenamide
SK 6882
Stepa
Tespa
Tespamin
Tespamine
Thiofozil
Thiophosphamide
Thiophosphoramide, *N,N',N"*-tri-1,2-ethanediyl-
Thiophosphoramide, *N,N',N"*-triethylene-
Thiophosphoramide, *N,N',N"*-triethylene-
Thiotef
Thio-Tep
Thio tepa
Thio-tepa S
Thiotriethylenephosphoramide
Tifosyl
N,N',N"-Triethylenethiophosphamide
Triethylene thiophosphoramide
N,N',N"-triethylenethiophosphoramide

Tio-tef	*N,N',N''*-Triethylenethiophosphoramide
Triaziridinylphosphine sulfide	Triethylenethiophosphorotriamide
N,N',N''-Tri-1,2-ethanediylphosphorothioic triamide	Tris(aziridinyl)phosphine sulfide
N,N',N''-Tri-1,2-ethanediylthiophosphoramide	Tris(1-aziridinyl)phosphine sulfide
Tri(ethyleneimino)thiophosphoramide	Tris(1-aziridinyl)phosphine sulphide
N,N',N''-Triethylenephosphorothioic triamide	Tris(ethylenimino)thiophosphate
N,N',N''-triethylenethiophosphamide	TSPA

1.2 Physical-Chemical Properties

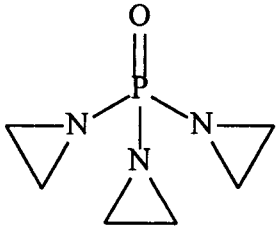
Property	Information	Reference
Color	White	Windholz (1983); Barnhart (1989); both cited by IARC (1990)
Physical State	crystals	Budavari (1996)
Melting Point, °C	51.5	Budavari (1996)
Solubility:		
Water at 25 °C	Soluble (19 g/100 mL)	Budavari (1996)
Organic Solvents	Freely Soluble in Alcohol Soluble in Benzene, Ether, and Chloroform	Budavari (1996)

1.3 Identification of Structural Analogues and Metabolites

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

Tris(1-aziridinyl)phosphine oxide (tepa) (CASRN 545-55-1)
Monoglutathionyl thiotepa
Diglutathionyl thiotepa
2-Aminoethyl glutathione

Tepa is extremely soluble in water, and soluble in alcohol, ether and acetone (Budavari, 1996). The structure of tepa is as follows:



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

Thiotepa was tested extensively for use as an intermediate in the manufacture of polymeric flame retardants for cotton, but it has probably not been used commercially in this way in the United States. Thiotepa has also been shown to be an effective insect chemosterilant, but problems associated with its application to insects, its toxicity, and its environmental effects have prevented its use in this way on a commercial basis. Investigation of the use of thiotepa in the treatment of leukemia is believed to have been the first trial in cancer therapy research (IARC, 1975). Thiotepa is a cytostatic agent that has been used in the treatment of lymphomas and a variety of solid tumors such as tumors of the breast and ovary. It has also been used in cases of urinary bladder malignancies, meningeal carcinomatosis, and various soft-tissue tumors (administration is by intravenous [i.v.] injection, or by intravesical or intracavitary instillation) (IARC, 1990; Reents, 1996). Treatment of meningeal carcinomatosis, however, now involves use of a reformulated preparation of thiotepa that is hypotonic and undiluted intrathecal use with this formulation has not been studied (Reents, 1996). The initial dosage of thiotepa is generally 5 to 40 mg (3 to 23 mg/m^2) at 1- to 4- weekly intervals. Doses up to 75 mg/m^2 have been used in children. High-dose therapy has involved daily doses in excess of 1100 mg/m^2. The dosage is generally adjusted on the basis of changes in leukocyte counts. Recently, thiotepa has been used at high doses in combination chemotherapy with cyclophosphamide in patients with refractory malignancies of the blood treated with autologous bone transplantation (IARC, 1990). Thiotepa was originally approved by the FDA in 1959. In 1995, a new lyophilized thiotepa powder with a longer shelf life was introduced (Reents, 1996).

2.2 Production

There is only one producer of thiotepa in the United States (Chem Sources, 1996), and no production data have been reported in appropriate online and print sources consulted in 1996 (see Appendix B). No data on imports or exports of thiotepa were available. One source is listed in the *Physicians' Desk Reference (1995).*

2.3 Environmental Exposure

Thiotepa is not a naturally occurring chemical. The primary routes of potential human exposure to thiotepa as a pharmaceutical are intravenous, intramuscular, and intrathecal administration; other parenteral routes such as intratumoral injections have also been used (IARC, 1990).

Potential exposure of health professionals may occur during the preparation and administration of the compound. 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 11,452 workers, including 8,724 women, were potentially exposed to thiotepa (NIOSH, 1984). This estimate was derived from observations of the use of the compound (41% of total observations) and tradename products (59%).

2.4 Regulations

EPA designates thiotepa as a hazardous constituent of wastes and regulates it under the Resource Conservation and Recovery Act (RCRA). EPA has not established a reportable quantity (RQ) for this compound under the Comprehensive Environmental Response,

Compensation, and Liability Act (CERCLA). FDA regulates thiotepa under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription drug. Thiotepa was originally approved by the FDA in 1959, with the warning that dosing must be adapted carefully. Labeling must identify the drug as a carcinogen. Since its original approval, numerous original or supplemental New Drug Applications (NDAs) have been released (Diogenes, 1976-1996). OSHA regulates thiotepa under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
F D A	Original FDA approval in 1959. 21 CFR 314 (3-29-74), prescription drug approval. 21 CFR 201.57 (6-29-79), labeling. New lyophilized powder with longer shelf life released in 1995 (Reents, 1996).	No reference given by Clinical Pharmacology Monograph: Thiotepa from Gold Standard Multimedia Inc. on the Internet.

3.0 HUMAN STUDIES

Summary: Thiotepa is *known to be a human carcinogen* based on studies in humans which indicates a causal relationship between exposure to thiotepa and human cancer (reviewed in IARC, 1990). Exposure to thiotepa is specifically associated with leukemia in humans. Adamson and Seiber (1981) summarized nine case reports from 1970 to 1978 of secondary development of nonlymphocytic leukemia occurring in cancer patients with primary cancers at other sites and who had received thiotepa as the only therapeutic agent. Additional evidence is found in a case-control study examining the development of leukemia as a secondary cancer in cancer patients undergoing chemotherapy compared to surgery alone. Patients undergoing chemotherapy were significantly more likely to develop secondary leukemia than those undergoing surgery alone, and in some of these patients, thiotepa was the only chemotherapeutic agent given (reviewed in IARC, 1990). More recent case reports or reviews were not found.

3.1 Studies Reviewed by Adamson and Seiber (1981)

Adamson and Seiber (1981) summarized case histories linking thiotepa exposure to development of acute nonlymphocytic leukemia (ANLL) reported in the literature from 1970 to 1978. In nine patients (one with bladder cancer, two with lung cancer, three with ovarian carcinoma, and three with breast cancer) receiving thiotepa as the only chemotherapeutic agent,

ANLL was observed (Rosner et al., 1978; cited by Adamson and Seiber, 1981; Kapadia and Krause, 1978; Reimer et al., 1977; Carey and Long, 1977; Perlman and Walker, 1973; Solomon and Firat, 1971; Garfield, 1970; Allan, 1970). Thiotepa used in combination with irradiation and chlorambucil or cyclophosamide in two patients and in combination with other drugs in three women with ovarian carcinoma also resulted in ANLL (Greenspan and Tung, 1974; cited by Adamson and Seiber, 1981; Kaslow et al., 1972).

3.2 Studies Reviewed by IARC (1990)

In a case-control study, 114 cases of leukemia that developed in patients previously diagnosed with ovarian cancer were compared with 342 controls with ovarian cancer who were matched by age, year of diagnosis of ovarian cancer, and survival duration (Kaldor et al., 1990; cited by IARC, 1990). A relative risk of 12 (95% confidence interval, 4.4-32) was associated with chemotherapy compared to treatment by surgery only. For 9/114 cases and 11/342 controls, thiotepa was the only chemotherapeutic used. Melphalan, chlorambucil, cyclophosphamide, and treosulphan (alkylating agents known to be carcinogenic [IARC, 1987]) were also found to be associated with significantly increased risks for leukemia (Kaldor et al., 1990; cited by IARC, 1990).

In two studies concluded by IARC (1990) to be too small to be informative, no increased risk of second malignancies was found among 470 patients with colorectoral cancer when randomized to low-dose (four doses of 0.2 mg/kg bw) adjuvant therapy with thiotepa and followed for a combined total of 3102 person-years (Boice et al., 1980; cited by IARC, 1990) or in 90 patients with breast cancer randomized to adjuvant therapy with thiotepa for 1 yr (0.8 mg/kg bw in divided doses followed by 0.2 mg/kg bw weekly maintenance) and followed for a combined total of 5819 person-years (Kardinal and Donegan, 1980; cited by IARC, 1990).

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 thiotepa in experimental animals (IARC, 1990). NCI (1978) concluded that under the conditions of its bioassay, thiotepa administered intraperitoneally (i.p.) was carcinogenic in B6C3F1 mice and Sprague-Dawley rats. In mice, thiotepa induced lymphoma or lymphocytic leukemia in both sexes and squamous-cell carcinoma of the skin and associated glands of males. In rats, thiotepa induced squamous-cell carcinoma of the skin or ear canal in both males and females and neoplasms of the hematopoietic system in males. Other rodent studies found thiotepa to be carcinogenic as well. The incidence of lung tumors in both male and female A/He mice was significantly increased by 4 weeks of intraperitoneal (i.p.) injections of thiotepa according to Stoner et al. (1973; cited by IARC, 1990). BR46 rats treated with thiotepa by intravenous (i.v.) injections once a week for 52 weeks developed benign and malignant tumors at multiple sites (Schmähl and Osswald, 1970, and Schmähl, 1975; cited by IARC, 1990).

4.1 Mice

Stoner et al. (1973; cited by IARC, 1990) induced lung tumors in A/He mice by injecting them i.p. with 95-99% pure thiotepa at doses of 1.6, 3.9, or 7.8 mg/kg bw/day in 0.1 mL purified tricaprylin (8.4-41.2 μmol/kg bw) for 3 days per week for 4 weeks, starting from 6 to 8 weeks of age. The mice were sacrificed 24 weeks after the first injection. The percentages of mice with lung tumors were 80%, 50%, and 55% in low-, mid-, and high-dose animals (males and females combined), respectively. The percentages of control mice with lung tumors were 28% in males and 20% in females. The multiplicity of lung adenomas was significantly increased in high-dose (1.50; $p < 0.001$) and mid-dose (0.74; $p < 0.05$) animals compared to male (0.24) and female (0.20) controls.

NCI (1978) reported that thiotepa induced increased incidences of malignant lymphoma and lymphocytic leukemia combined in the high-dose mice when NCI conducted a carcinogenesis bioassay with B6C3F1 mice using 98.0 ± 1.0% pure thiotepa at i.p. doses of 1.15 or 2.3 mg/kg bw (6.08 or 12.1 μmol/kg bw), 3 times per week for up to 52 weeks. All high-dose females had died by 43 weeks and all high-dose males had died by 56 weeks. The study was terminated during weeks 86-87. At this time, 15/35 low-dose males, 17/35 low-dose females, 7/15 vehicle control males, and 12/15 vehicle control females were still alive. The vehicle control received phosphate-buffered saline. Pooled vehicle controls were also used; that is, the results of control mice in another bioassay were included with matched vehicle controls. Because of the early deaths, statistical analyses were based on incidences of tumors in mice that survived at least 52 weeks unless a tumor was observed before week 52.

The combined incidences of malignant lymphoma and lymphocytic leukemia were significantly greater in high-dose mice (32/32 females, 26/28 males; $p < 0.001$, Fisher's exact test) compared to vehicle (0/14 females, 1/8 males) and pooled vehicle (0/29 females, 1/18 males) controls. IARC (1990) noted the high death rate of high-dose mice and that the NCI (1978) study used controls pooled from different studies.

The summary of NCI (1978) stated that when the incidences of low-dose male mice with tumors appearing at other sites (squamous-cell carcinomas of the skin of 7 low-dose males, of the preputial glands of 6, and of the ear canal of 2 mice) were combined (14/24), the results were statistically significant when compared to the vehicle controls (0/8; $p = 0.004$) or the pooled controls (0/18; $p < 0.001$). In addition, 1 of 2 high-dose mice surviving beyond week 52 had a carcinoma of the preputial gland.

4.2 Rats

Schmähl and Osswald (1970; cited by IARC, 1990) and Schmähl (1975; cited by IARC, 1990) reported on a study in BR46 rats in which thiotepa of unspecified purity induced malignant tumors at multiple sites. The rats were treated from age 100 days with 1 mg/kg bw (5.3 μmol/kg bw; vehicle not specified) intravenously (i.v.), once per week for 2 weeks. Thirty of 48 treated male rats were still alive when the first tumor was detected (induction time not specified), compared to 65 controls. Malignant tumors were detected in 9/30 treated rats (2 sarcomas in the abdominal cavity, 1 lymphosarcoma, 1 'myelosis', 1 seminoma, 1 fibrosarcoma and 1 hemangioendothelioma of the salivary gland, 1 mammary sarcoma, 1 pheochromocytoma) and in 4/65 untreated controls (3 mammary sarcomas, 1 pheochromocytoma). Benign tumors were

detected in 5/30 treated animals and in 3/65 untreated controls. IARC (1990) noted the short latency of tumor induction.

NCI (1978) reported multi-site tumors induced in Sprague-Dawley rats (35-, 42-, or 58-day-old at the beginning of the study) treated i.p. with 98.0 ± 1.0% pure thiotepa at doses of 0.7, 1.4, or 2.8 mg/kg bw (3.7-14.8 μmol/kg bw), 3 times per week for up to 52 weeks. The low-dose group was started 69 weeks after the beginning of the original study, together with additional control groups because of deaths in the mid-dose and high-dose groups. Details about the control groups are presented in Table 4-1. Pooled control groups were used for statistical comparisons (Fisher exact test).

All high-dose males had died by week 19 and all high-dose females had died by week 21. Treatment of mid-dose animals was terminated at week 34, and these animals were observed until weeks 78-81, at which time all had died. All other groups were observed until weeks 82-87. As described above for mice, statistical analyses were based on time-adjusted incidences of tumors because of the early deaths (NCI, 1978; IARC, 1990).

Thiotepa induced an increased incidence of lymphoma and leukemia combined in low- and mid-dose males. Malignant lymphomas, lymphocytic leukemia, and granulocytic leukemia were detected in 6/34 low-dose ($p = 0.020$; pooled controls, 0/29) and 6/16 mid-dose ($p < 0.001$; pooled controls, 0/30) males (NCI, 1978; IARC, 1990).

Thiotepa induced an increased incidence of uterine adenocarcinomas in mid-dose female rats (7/21 vs. pooled controls incidence of 0/28; $p = 0.001$) (NCI, 1978; IARC, 1990). Two low-dose females had such tumors; the concurrent controls had none (NCI, 1978; IARC, 1990). The results should be considered equivocal in that although NCI (1978) concluded the data "suggest a positive association" with thiotepa administration, NCI stopped short of concluding that this is a carcinogenic effect.

The incidence of adenocarcinomas of the mammary gland was significantly increased in 8/24 mid-dose females ($p = 0.006$ using pooled controls, 1/28), but were also found in 3/10 untreated concurrent controls and 1 pooled control (NCI, 1978). NCI (1978, p. 46) concluded that these tumors could not "be clearly related to administration of the test chemical."

Male rats of the low- and mid-dose groups and mid-dose females showed significantly increased incidences of squamous-cell carcinoma of the skin or ear canal. The incidence in the low-dose males was 7/33 compared to 0/29 in the pooled controls ($p = 0.009$); in the mid-dose males, the incidence was 3/13 compared to 0/30 in the pooled controls ($p = 0.023$). The incidence in the mid-dose females was 8/21 compared to 0/28 in the pooled controls ($p < 0.001$). Two low-dose female rats had such tumors whereas none appeared in the concurrent controls for the low-dose rats.

The results for tumors in the cranial cavity were also equivocal. The incidences of neuroepitheliomas or nasal carcinomas were not significantly increased in treated animals (3 in low-dose males, 2 in low dose females, 2 in mid-dose females), but these tumors were not detected in corresponding controls, in pooled vehicle controls, or in 380 Sprague-Dawley control rats of each sex in other bioassays at the same laboratory (NCI, 1978; IARC, 1990). NCI (1978, p.47) concluded that these uncommon tumors "may be associated with administration of the chemical."

Table 4-1. Mammalian Carcinogenicity Studies of Thiotepa

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)	80M, 80F (0.1 mL tricaprylin alone)	thiotepa, 95-99% pure	1.6, 3.9, or 7.8 mg/kg bw/day in 0.1 mL purified tricaprylin (8.4–41.2 μmol/kg bw), 3 days/wk Total doses 19, 47, or 94 mg/kg bw (100 - 490 μmol/kg bw)	4 wk	Animals were killed 24 weeks after the first injection. **Lung:** Positive (for tumorigenesis) The percentages of mice with lung tumors were 80%, 50%, and 55% in low-, medium-, and high-dose animals (males and females combined), respectively. Lung tumor percentages in controls were 28% in males and 20% in females. The multiplicity of lung adenomas was significantly increased in high-dose (1.50) and mid-dose (0.74) animals compared to male (0.24) and female (0.20) controls. IARC did not specify which statistical test was used.	Stoner et al. (1973; cited by IARC, 1990)

Table 4-1. Mammalian Carcinogenicity Studies of Thiotepa (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old B6C3F1 mice	35M, 35F (for both doses)	*untreated controls*: 15M, 15F *matched vehicle controls* (phosphate buffered saline alone): 15M, 15F *pooled vehicle controls:* 30M, 30F (15M, 15F from a bioassay on another chemical plus the matched vehicle controls)	thiotepa, 98.0 ± 1.0% pure	1.15 or 2.3 mg/kg bw (6.08 or 12.1 µmol/kg bw), 3 times/wk	up to 52 wk	All high-dose females had died by 43 weeks and all high-dose males had died by 56 weeks. The study was terminated during weeks 86-87. At this time, 15/35 low-dose males, 17/35 low-dose females, 7/15 vehicle control males, and 12/15 vehicle control females were still alive. Statistical analyses were based on time-adjusted incidences of tumors because of the early deaths. Animals without tumors that died before week 52 were excluded from the statistical analyses. **Lymphatic System:** Positive (for lymphoma and leukemia) The incidences of malignant lymphoma and lymphocytic leukemia combined were significantly greater in high-dose animals (32/32 females, 26/28 males; $p < 0.001$, Fisher's exact test) compared to matched vehicle (0/14 females, 1/8 males) and pooled vehicle (0/29 females, 1/18 males) controls. **Other sites:** Positive (when combined in low-dose males) The combined incidence (14/24) of squamous-cell carcinomas of the skin in 7 low-dose males, of the preputial glands of 6, and of the ear canal of 2 was statistically significant when compared to the vehicle controls (0/8; $p = 0.004$) or the pooled controls (0/18; $p < 0.001$). One of two high-dose mice surviving beyond week 52 had a carcinoma of the preputial gland. IARC noted the high death rate of high-dose animals and that the study used controls pooled from different studies.	NCI (1978); IARC (1990)

Table 4-1. Mammalian Carcinogenicity Studies of Thiotepa (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Intravenous Injection*							
100-day-old BR46 rats	48M	89M (untreated)	thiotepa, purity not specified	1 mg/kg bw (5.3 µmol/kg bw; vehicle not specified), once/wk	52 wk	Thirty treated animals were still alive when the first tumor was detected (induction time not specified), compared to 65 controls. **Malignant Tumors:** Malignant tumors were detected in 9/30 treated animals (2 sarcomas in the abdominal cavity, 1 lymphosarcoma, 1 'myelosis', 1 seminoma, 1 fibrosarcoma, and 1 hemangioendothelioma of the salivary gland, 1 mammary sarcoma, 1 pheochromocytoma) and in 4/65 controls (3 mammary sarcomas, 1 pheochromocytoma) ($p < 0.01$). The statistical test for significance was not specified. **Benign Tumors:** Benign tumors were detected in 5/30 treated animals and in 3/65 controls. IARC noted the short latency of tumor induction.	Schmähl and Osswald (1970; cited by IARC, 1990) Schmähl (1975; cited by IARC, 1990)

Table 4-1. Mammalian Carcinogenicity Studies of Thiotepa (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Intraperitoneal Injection*							
35- (MD & HD M), 42- (MD & HD F), or 58-day-old (LD M & F) Sprague-Dawley rats The LD group was started 69 weeks after the beginning of the original study, together with 2 additional controls groups because of deaths in the MD & HD groups.	35-39M, 31-35F (for each dose)	*untreated controls*: Two groups of 10M, 10F, 1 concurrent with the MD & HD groups & 1 concurrent with the LD group *matched vehicle controls*: Two concurrent groups of 10M, 10F (buffered saline alone; 2.5 mL/kg bw) *pooled vehicle controls*: Three groups of 10M, 10F (the concurrent thiotepa vehicle controls plus controls from bioassays on 2 other chemicals)	thiotepa, 98.0 ± 1.0% pure	0.7, 1.4, or 2.8 mg/kg bw (3.7–14.8 µmol/kg bw), 3 times/wk, injected i.p.	up to 52 wk	All high-dose males had died by week 19 and all high-dose females had died by week 21. Treatment of mid-dose animals was terminated at week 34, and these animals were observed until weeks 78-81, at which time all had died. All other groups were observed until weeks 82-87. Statistical analyses were based on time-adjusted incidences of tumors because of the early deaths. Animals without tumors that died before week 52 were not included in the statistical analyses. Pooled control groups were used for statistical comparisons. Fisher's exact test was used. **Lymphatic System and Blood:** Positive (for lymphoma and leukemia combined; males only) Malignant lymphomas, lymphocytic leukemia, and granulocytic leukemia were detected in 6/34 low-dose ($p = 0.020$; pooled controls, 0/29) and 6/16 mid-dose ($p < 0.001$; pooled controls, 0/30) males. **Uterus:** Equivocal (for adenocarcinomas) Uterine adenocarcinomas were detected in 7/21 mid-dose ($p= 0.001$; pooled controls, 0/28) and 2/29 low-dose females whereas none was observed in the concurrent untreated controls. Although NCI (1978; p. 35) concluded the data "suggested a positive association," they stopped short of concluding that this is a carcinogenic effect.	NCI (1978); IARC (1990)

Table 4-1. Mammalian Carcinogenicity Studies of Thiotepa (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats, i.p. injection (continued)						**Mammary Gland:** Suggestive (for adenocarcinomas) Adenocarcinomas were detected in 8/24 mid-dose females ($p = 0.006$; pooled controls, 1/28). However, adenocarcinomas were also detected in 3/10 low-dose concurrent untreated controls and in one low-dose pooled control. NCI (1978) concluded that these tumors could not "be clearly related to administration of the test chemical." **Integument:** Positive (for squamous-cell carcinoma of the skin or ear canal) LD M (7/33 vs. 0/29 in the pooled controls; $p = 0.009$) & MD M (3/13 vs. 0/30; $p = 0.023$) and MD F (8/21 Vs 0/28; $p < 0.001$) showed significantly increased incidences of squamous-cell carcinoma of the skin or ear canal. Two LD F had such tumors whereas none appeared in the LD concurrent controls. **Cranial Cavity:** Equivocal The incidences of neuroepitheliomas or nasal carcinomas were not significantly increased in treated animals (3 in low-dose males, 2 in low-dose females, 2 in mid-dose females), but these tumors were not detected in corresponding controls, in pooled vehicle controls, or in 380 Sprague-Dawley control rats of each sex in other bioassays at the same laboratory. NCI (1978) concluded that these uncommon tumors "may be associated with administration of the chemical." IARC noted the high death rate among high- and medium-dose animals and that this necessitated the later inclusion of the lower dose-treated group. IARC also noted that the study used controls pooled from different studies.	NCI (1978); IARC (1990) (concluded)

5.0 GENOTOXICITY

Studies of the genotoxic effects of thiotepa are summarized in Table 5-1 [see also Figure 5-1 Genetic Activity Profile (Data limited to IARC, 1990)]. Unless otherwise noted, rat liver S9 was the source of metabolic activation *in vitro*. In addition, for the sake of simplicity, multiple citations in IARC for the same genetic toxicity assay were discussed as a group rather than individually. Because of the extensive information available in IARC (1990) on the *in vitro* and *in vivo* genotoxicity of thiotepa, the post-1990 genotoxicity literature selection strategy was limited solely to studies that might offer additional unique information. No such studies were identified.

Summary: Thiotepa was found to exhibit highly reproducible genotoxicity in a wide variety of prokaryotic, lower eukaryotic, and mammalian *in vitro* and *in vivo* test systems. When tested *in vitro* in the absence of S9, thiotepa was found to induce gene mutations in *Salmonella typhimurium, Aspergillus nidulans, Drosophila melanogaster, Aedes aegypti,* and Chinese hamster V79 cells; unscheduled DNA synthesis (UDS) in human lymphocytes; sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells and mouse, monkey, and human lymphocytes; chromosomal aberrations in *Vicia faba,* CHO cells, and rabbit, monkey, and human lymphocytes; and cell transformation in mouse C3H 10T1/2 cells. *In vivo*, it was positive for dominant lethal mutations in male mice and for *tk* mutations in mouse lymphoma cells (host-mediated assay); SCE in mouse bone marrow and monkey lymphocytes; chromosomal aberrations in mouse bone marrow, liver cells, and spermatocytes, and in rabbit, monkey, and human lymphocytes; micronucleated erythrocytes in mouse and rat bone marrow; and heritable translocations and sperm abnormalities in mice.

5.1 Prokaryotic Systems

As reviewed by IARC (1990), 2 papers reported that thiotepa induced reverse gene mutations in *S. typhimurium* strains TA1535 (Benedict et al., 1977), and TA100 (Pak et al., 1979) in the absence of metabolic activation [LED = 100 μg/plate (0.52 μmol/plate)]. In TA98, contradictory results were obtained. In two host-mediated assays in mice cited by IARC (1990), thiotepa was mutagenic to *S. typhimurium* strains TA1535 (Arni et al., 1977) and G46 (Devi and Reddy, 1980).

5.2 Lower Eukaryotic Systems

Bignami et al. (1982; cited by IARC, 1990) found that thiotepa induced 8-azaguanine resistant forward mutations in the fungus *A. nidulans* [LED = 12.5 μg/plate (0.07 μmol/plate)]. Two papers cited by IARC (1990) stated that in *D. melanogaster,* thiotepa induced sex-linked recessive mutations [LED = 0.23 g/mL (1.2 M)]. Rodriguez and Rodriguez (1985; cited by IARC, 1990) reported that thiotepa induced dominant lethal mutations in the mosquito *A. aegypti* (dose levels not provided). IARC (1990) cited one paper (Kihlman, 1975) where thiotepa induced SCE [LED = 37.8 g/mL (200 M)] and three papers where it induced chromosomal aberrations [LED = 19 g/mL (100 M)] in the root meristem cells of fava beans, *V. faba.*

5.3 Mammalian Systems *In Vitro*

5.3.1 DNA Damage

Titenko (1983; cited by IARC, 1990) reported that thiotepa induced unscheduled DNA synthesis (UDS) in unstimulated human peripheral blood lymphocytes in the absence of S9 activation [LED = 1.0 g/mL (5.3 M)].

IARC (1990) reviewed three papers that showed thiotepa to induce SCE in CHO cells [LED = 0.05 g/mL (0.3 M)] in the absence of metabolic activation. Anderson et al. (1987; cited by IARC, 1990) found that thiotepa induced SCE in mouse cells (cell type not specified) in the absence of S9 activation [LED = 0.2 g/mL (1 M)]. In addition, Kuzin et al. (1987; cited by IARC, 1990) reported that thiotepa induced SCE in rhesus monkey peripheral blood lymphocytes without S9 [LED = 2.5 g/mL (13 M)]. IARC (1990) reviewed 5 papers that showed thiotepa induced SCE in human peripheral blood lymphocytes [LED = 0.03 g/mL (0.2 M)], tested only in the absence of metabolic activation.

5.3.2 Gene Mutations

Paschin and Kozachenko (1982; cited by IARC, 1990) reported that thiotepa induced mutations at the *hprt* locus in Chinese hamster lung V79 cells in the absence of metabolic activation [LED = 2 g/mL (11 M)].

5.3.3 Chromosomal Damage

IARC (1990) cited 3 studies in which thiotepa induced chromosomal aberrations in CHO cells without metabolic activation [LED = 2.0 g/mL (11 M)]. Bochkov et al. (1982; cited by IARC, 1990) found that chromosomal aberrations were induced by thiotepa in rabbit peripheral blood lymphocytes without S9 activation [LED = 5.0 g/mL (26 M)]. In addition, Kuzin et al. (1987; cited by IARC, 1990) reported that thiotepa induced chromosomal aberrations in rhesus monkey peripheral blood lymphocytes without S9 [LED = 2.5 g/mL (13 M)]. IARC (1990) also cited 11 studies in which thiotepa induced chromosomal aberrations in human peripheral blood lymphocytes in the absence of metabolic activation [LED = 1.0 g/mL (5.3 M)].

5.3.4 Morphological Transformation

Benedict et al. (1977; cited by IARC, 1990) stated that mouse C3H 10T1/2 cells treated with thiotepa exhibited increased levels of morphological transformation [LED = 0.1 g/mL (0.5 M)].

5.4 Mammalian Systems *In Vivo*

5.4.1 DNA Damage

Anderson et al. (1983; cited by IARC, 1990) reported that thiotepa administered i.p. induced SCE in the bone marrow cells of mice (strain not provided) [LED = 2.0 mg/kg (11 μmol/kg)]. Kuzin et al. (1987; cited by IARC, 1990) also reported that thiotepa administered i.v. induced sister chromatid exchanges in the peripheral blood lymphocytes of rhesus monkeys [LED = 3.0 mg/kg (16 μmol/kg)].

5.4.2 Gene Mutations

IARC (1990) reported on 5 papers that stated thiotepa administered i.p. to male mice (strain not provided) induced dominant lethal mutations in spermatogonia and spermatocytes [LED = 0.2 mg/kg (1 μmol/kg)]. In addition, in a host-mediated assay (Lee et al., 1973; cited by IARC, 1990), thiotepa administered subcutaneously (s.c.) to AKD_2F_1 mice induced a significant increase in mutations at the *tk* locus in inoculated mouse lymphoma L5178Y cells [LED = 7.5 mg/kg (40 μmol/kg)].

5.4.3 Chromosomal Damage

IARC (1990) reviewed 4 papers in which thiotepa induced chromosomal aberrations in mouse bone marrow cells [LED = 0.32 mg/kg (1.7 μmol/kg)] and 3 papers in which thiotepa induced chromosomal aberrations in mouse spermatocytes [LED = 1.0 mg/kg (5.3 μmol/kg)]. Korogodina and Lil'p (1978, cited by IARC, 1990) found chromosomal aberrations in the liver cells of mice (strain not provided) treated with thiotepa [LED = 8.0 mg/kg (42 μmol/kg)]. Bochkov et al. (1982; cited by IARC, 1990) further reported that thiotepa induced chromosomal aberrations in the lymphocytes of treated rabbits [LED = 3.0 mg/kg (16 μmol/kg)]. Kuzin et al. (1987; cited by IARC, 1990) found chromosomal aberrations in the peripheral blood lymphocytes of rhesus monkeys treated with thiotepa [LED = 3.0 mg/kg (16 μmol/kg)]. Finally, Selezneva and Korman (1973; cited by IARC, 1990) stated that thiotepa induced aberrations in the chromosomes of peripheral blood lymphocytes sampled from 5 human subjects treated intramuscularly (i.m.) for 10 days and sampled 24 h after the last treatment [LED = 0.14 mg/kg (0.74 μmol/kg)].

IARC (1990) reviewed 4 papers in which thiotepa induced micronuclei in bone marrow polychromatic erythrocytes (PCE) of mice [LED = 1.0 mg/kg (5.3 μmol/kg)]. Setnikar et al. (1976; cited by IARC, 1990) reported that thiotepa also induced bone marrow micronucleated PCE in rats [LED = 4.0 mg/kg (21 μmol/kg)] (strain not provided). IARC (1990) cited 4 papers that reported thiotepa to induce heritable translocations in male mice (strain not provided) treated i.p. with thiotepa [LED = 1.25 mg/kg (6.61 μmol/kg)].

5.4.4 Sperm Abnormalities

Bruce and Heddle (1979; cited by IARC, 1990) reported that male mice (species not stated) treated intravenously (i.v.) with thiotepa exhibited increased levels of sperm abnormalities [LED = 2.5 mg/kg (13 μmol/kg)].

Table 5-1. Summary of Thiotepa Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Prokaryotic Systems							
Salmonella typhimurium strains TA98, TA100, and TA1535	*his* reverse gene mutations	-	n.p.	n.g.	positive	LED = 100 µg/plate (0.52 µmol/plate)	3 papers cited by IARC (1990)
S. typhimurium strain TA1535	*his* reverse gene mutations (host-mediated assay)	NA	n.p.	n.g.	positive	LED = 12.4 µg/kg (65.5 µmol/kg)	Arni et al. (1977; cited by IARC, 1990)
S. typhimurium strain G46	*his* reverse gene mutations (host-mediated assay)	NA	n.p.	n.g.	positive	LED = 2.5 µg/plate (13.2 µmol/plate)	Devi and Reddy (1980; cited by IARC, 1990)
5.2 Lower Eukaryotic Systems							
Aspergillus nidulans	8-azaguanine resistant forward gene mutations	-	n.p.	n.g.	positive	LED = 12.5 µg/plate (0.07 µmol/plate)	Bignami et al. (1982; cited by IARC, 1990)
Drosophila melanogaster	sex-linked recessive mutations	-	n.p.	n.g.	positive	LED = 0.23 µg/mL (1.2 µM)	2 papers cited by IARC (1990)
Aedes aegypti (mosquito)	dominant lethal mutations	-	n.p.	n.g.	positive	Information on LED not provided	Rodriguez and Rodriguez (1985; cited by IARC, 1990)
Vicia faba root meristem cells	sister chromatid exchanges (SCE)	-	n.p.	n.g.	positive	LED = 37.8 µg/mL (200 µM)	Kihlman (1975; cited by IARC, 1990)

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

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
V. faba root meristem cells	chromosomal aberrations	-	n.p.	n.g.	positive	LED = 19.0 µg/mL (100 µM)	3 papers cited by IARC (1990)
5.3 Mammalian Systems *In Vitro*							
5.3.1 DNA Damage							
human peripheral blood lymphocytes (unstimulated)	unscheduled DNA synthesis (UDS)	-	n.p.	n.g.	positive	LED = 1.0 µg/mL (5.3 µM)	Titenko (1983; cited by IARC, 1990)
Chinese hamster ovary (CHO) cells	SCE	-	n.p.	n.g.	positive	LED = 0.05 µg/mL (0.3 µM)	3 papers cited by IARC (1990)
mouse cells (species not provided)	SCE	-	n.p.	n.g.	positive	LED = 0.2 µg/mL (1 µM)	Anderson (1983; cited by IARC, 1990)
rhesus monkey lymphocytes	SCE	-	n.p.	n.g.	positive	LED = 2.5 µg/mL (13 µM)	Kuzin et al. (1987; cited by IARC, 1990)
human peripheral blood lymphocytes	SCE	-	n.p.	n.g.	positive	LED = 0.03 µg/mL (0.2 µM)	5 papers cited by IARC (1990)
5.3.2 Gene Mutations							
Chinese hamster lung V79 cells	*hprt* gene mutations	-	n.p.	n.g.	positive	LED = 2.0 µg/mL (11 µM)	Paschin & Kozachenko (1982; cited by IARC, 1990)

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

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.3.3 Chromosomal Damage							
CHO cells	chromosomal aberrations	-	n.p.	n.g.	positive	LED = 2.0 μg/mL (11 μM)	3 papers cited by IARC (1990)
rabbit lymphocytes (species not provided)	chromosomal aberrations	-	n.p.	n.g.	positive	LED = 5.0 μg/mL (26 μM)	Bochkov et al. (1982; cited by IARC, 1990)
rhesus monkey peripheral blood lymphocytes	chromosomal aberrations	-	n.p.	n.g.	positive	LED = 2.5 μg/mL (13 μM)	Kuzin et al. (1987; cited by IARC, 1990)
human peripheral blood lymphocytes	chromosomal aberrations	-	n.p.	n.g.	positive	LED = 1.0 μg/mL (5.3 μM)	11 papers cited by IARC (1990)
5.3.4 Morphological Transformation							
C3H 10T1/2 mouse cells	morphological transformation	-	n.p.	n.g.	positive	LED = 0.1 μg/mL (0.5 μM)	Benedict et al. (1977; cited by IARC, 1990)
5.4 Mammalian Systems *In Vivo*							
5.4.1 DNA Damage							
mouse (strain not provided)	SCE in bone marrow cells	NA	n.p.	n.g.	positive	LED = 2.0 mg/kg (11 μmol/kg) i.p.	Anderson (1983; cited by IARC, 1990)
rhesus monkey	SCE in peripheral blood lymphocytes	NA	n.p.	n.g.	positive	LED = 3.0 mg/kg (16 μmol/kg) i.v.	Kuzin et al. (1987; cited by IARC, 1990)

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

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
5.4.2 Gene Mutations							
male mice (strain not provided)	dominant lethal mutations	NA	n.p.	n.g.	positive	LED = 0.2 mg/kg (1 μmol/kg) i.p.	5 papers cited by IARC (1990)
mouse lymphoma L5178Y cells in AKD_2F_1 mice	*tk* gene mutations (host-mediated assay)	NA	n.p.	n.g.	positive	LED = 7.5 mg/kg (40 μmol/kg) s.c.	Lee (1973; cited by IARC, 1990)
5.4.3 Chromosomal Damage							
mouse (strain not provided)	chromosomal aberrations in bone marrow cells	NA	n.p.	n.g.	positive	LED = 0.32 mg/kg (1.7 μmol/kg) i.p.	4 papers cited by IARC (1990)
mouse (strain not provided)	chromosomal aberrations in spermatocytes	NA	n.p.	n.g.	positive	LED = 1.0 mg/kg (5.3 μmol/kg) p.o.	3 papers cited by IARC (1990)
mouse (strain not provided)	chromosomal aberrations in liver cells	NA	n.p.	n.g.	positive	LED = 8.0 mg/kg (42 μmol/kg) i.p.	Korogodina & Lil'p (1978; cited by IARC, 1990)
rabbit (strain not provided)	chromosomal aberrations in lymphocytes	NA	n.p.	n.g.	positive	LED = 3.0 mg/kg (16 μmol/kg) i.v.	Bochkov et al. (1982; cited by IARC, 1990)
rhesus monkey	chromosomal aberrations in lymphocytes	NA	n.p.	n.g.	positive	LED = 3.0 mg/kg (16 μmol/kg) i.v.	Kuzin et al. (1987; cited by IARC, 1990)
human	chromosomal aberrations in peripheral blood lymphocytes	NA	n.p.	n.g.	positive	5 patients treated for 10 days and sampled 24 h after last treatment, LED = 0.14 mg/kg (0.742 μmol/kg) i.m.	Selezneva & Korman (1973; cited by IARC, 1990)

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

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
mouse (strain not provided)	micronuclei in bone marrow polychromatic erythrocytes	NA	n.p.	n.g.	positive	LED = 1.0 mg/kg (5.3 μmol/kg) i.p.	4 papers cited by IARC (1990)
rat (strain not provided)	micronuclei in bone marrow polychromatic erythrocytes	NA	n.p.	n.g.	positive	LED = 4.0 mg/kg (21 μmol/kg) i.p.	Setnikar et al. (1976; cited by IARC, 1990)
mouse (strain not provided)	heritable translocations	NA	n.p.	n.g.	positive	LED = 1.25 mg/kg (6.61 μmol/kg) i.p.	4 papers cited by IARC (1990)
5.4.4 Sperm Abnormalities							
mouse (strain not provided)	sperm morphology	NA	n.p.	n.g.	positive	LED = 2.5 mg/kg (13 μmol/kg) i.v.	Bruce and Heddle (1979; cited by IARC, 1990)

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

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

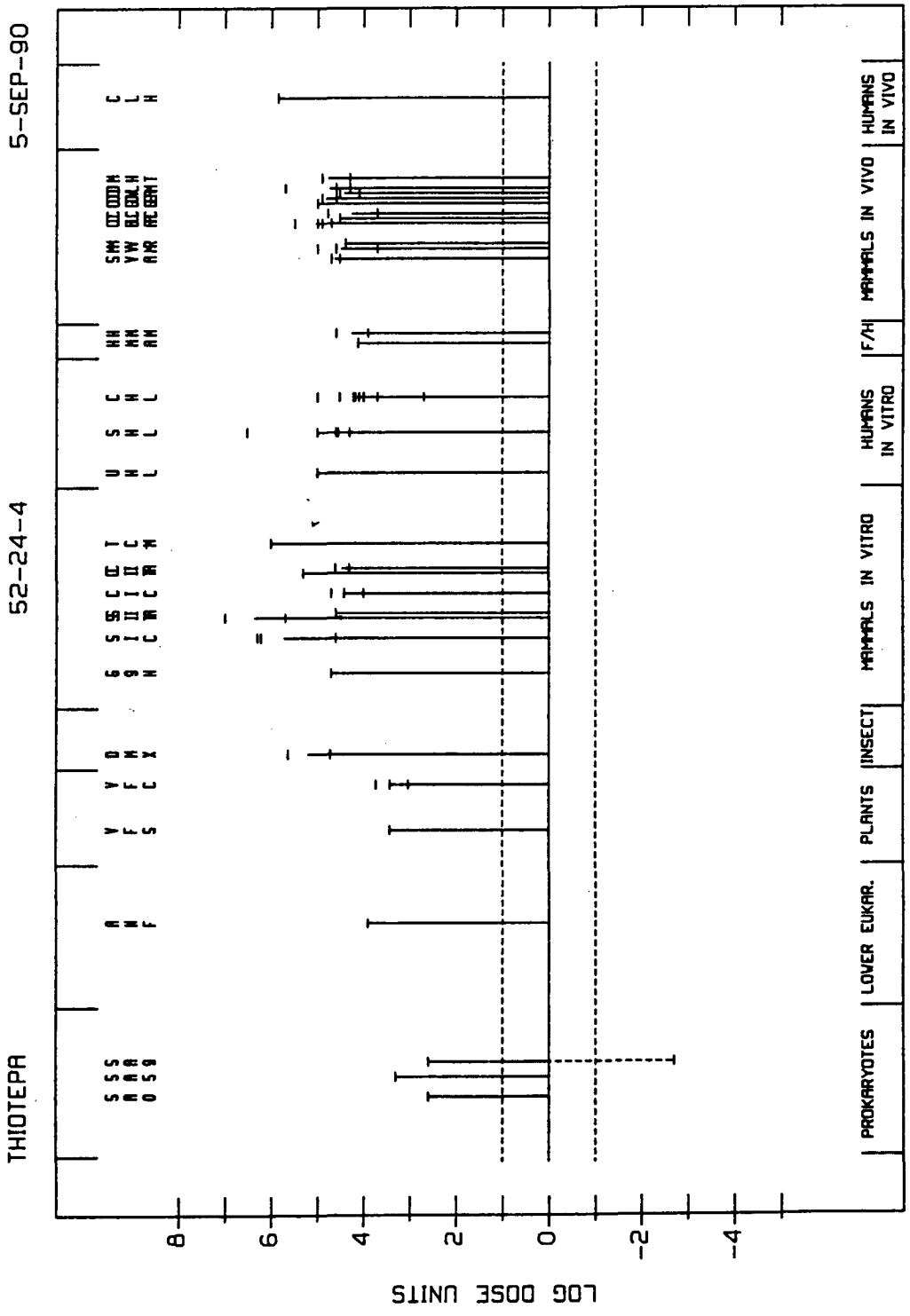
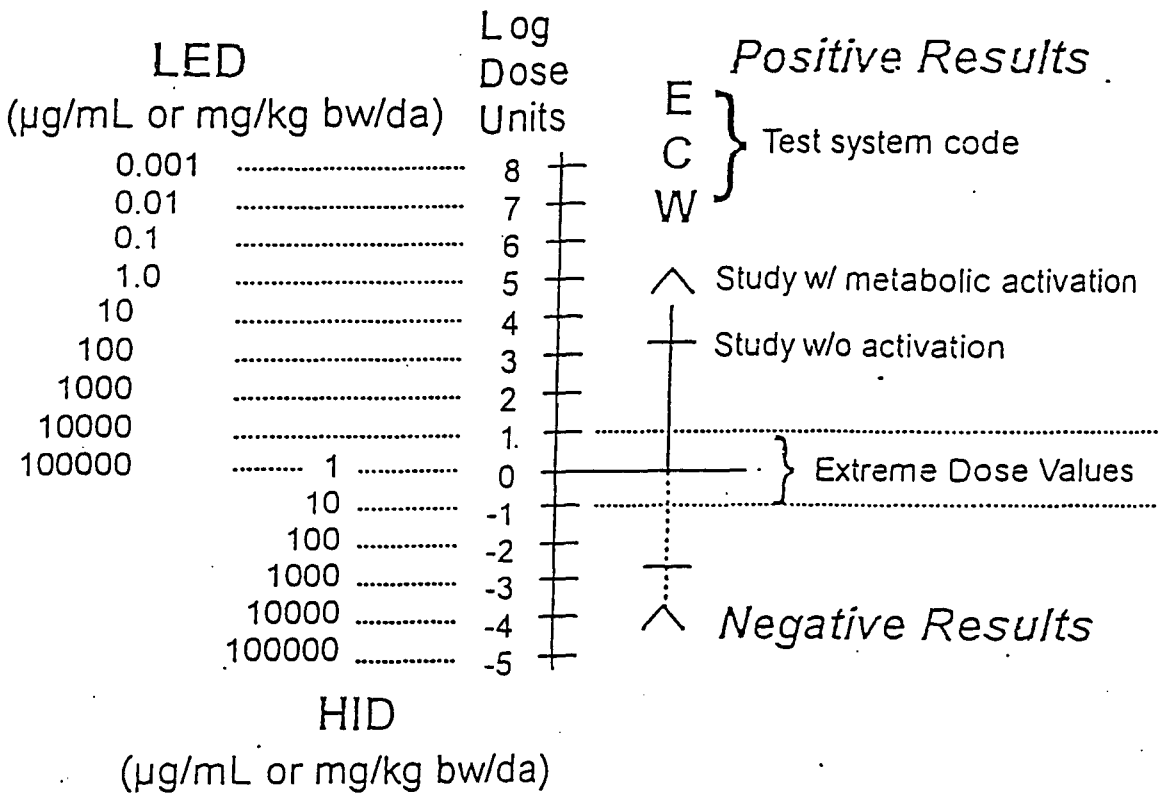


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



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: Thiotepa is an alkylating agent containing three aziridine (ethylenimine) moieties and a four-coordinated phosphorus atom. Thiotepa is rapidly absorbed and distributed to lungs, kidneys, heart, plasma, and other organs of rodents. In humans, absorption of thiotepa after oral administration was erratic and incomplete, and was thought to be due to acid instability. It is metabolized primarily by liver (cytochrome P-450 isozymes) to tepa (major oxidative metabolite) and/or conjugated by glutathione to form monoglutathionyl or diglutathionyl thiotepa. Thiotepa can also be conjugated via nonenzymatic reaction with glutathione at low pH.

The absorption, distribution, metabolism, and excretion of thiotepa have been reviewed by Le Blanc and Waxman (1989), IARC (1990), Lind and Ardiet (1993), and Dirven et al. (1996) and are discussed below. Experimental details of the studies discussed in this section and metabolite identification are presented in Table 6-1. Structures of many of the metabolites are shown in the metabolic pathways depicted in Figure 6-1.

6.1.1 Absorption, Distribution, and Excretion

A study conducted by Boone et al. (1962, cited by IARC, 1990) showed that 5 min after i.v. or intraarterial injection of radiolabeled thiotepa in Sprague-Dawley rats, slightly higher concentrations of radiolabel were found in lungs, kidneys, heart, and plasma when compared to other organs. Within 8.5 h, 94-98% of radiolabel administered i.v. was excreted in urine. Tepa [tris(1-aziridinyl)phosphine oxide] accounted for approximately 30% of the urinary radioactivity; most (percent not given) of the radioactivity was associated with parent compound (Boone et al., 1962; cited by IARC, 1990).

Thiotepa injected i.p. at 9.3 mg/kg bw (49 μmol/kg bw) into Sprague-Dawley rats was found in plasma (5.4%), peritoneal fluid (26%), urine (1.9%), kidney (0.7%), muscle (25.9%), lung (0.6%), and liver (3.8%) 1 h after administration of radiolabeled drug (Litterst et al., 1982, cited by IARC, 1990).

In mice and rats 9 days after an i.v. injection of ^{32}P-thiotepa, low levels of radioactivity were detected in most tissues, with higher levels detected in the blood of rats (Craig et al., 1959; cited by IARC, 1990).

Following an i.v. dose of radiolabeled thiotepa to female mongrel dogs, 75-85% of the radioactivity was recovered in urine (time elapsed not given), with 0.2-0.3% detected as parent compound (Mellet et al., 1962; cited by IARC, 1990). Subsequent studies conducted by Mellet and Woods (1960; cited by IARC, 1990) showed that i.v. (3 mg/kg bw [16 μmol/kg bw]) or oral (6 mg/kg bw [32 μmol/kg bw]) administration of thiotepa to dogs resulted in approximately 13% of the dose excreted as tepa (time elapsed not given). Two hours after i.v. injection of thiotepa, the plasma level of tepa was approximately 1.2 μg/mL (6.3 μM). Mellet and Woods (1960; cited by IARC, 1990) found that 50% of the [presumably orally; monograph not clear] administered thiotepa was absorbed.

Hagen and Nelson (1987; cited by IARC, 1990) found that approximately 10% of thiotepa added to sera from patients and healthy individuals was bound to protein.

6.1.2 Cellular Transport and Accumulation

Egorin et al. (1989) studied the cellular transport and accumulation of thiotepa in L1210 murine lymphoblastic leukemic cells and human red blood cells (RBCs) incubated with [^{14}C]thiotepa. As suggested by an octanol:PBS partition coefficient of 2.4 $\pm$ 0.1 (n = 8), thiotepa proved to be lipophilic. Obtaining this value enabled Erogin et al. (1989) to calculate the approximate permeability coefficient (P_s; 2.82 x 10^{-4} to 1.81 x 10^{-3}) of thiotepa and subsequently to make an estimation of the $t_{1/2}$ of accumulation of thiotepa in L1210 cells (0.063 and 0.40 s). As predicted by the estimated $t_{1/2}$, accumulation of ^{14}C in L1210 cells was rapid, was expressed as a biphasic process, and was essentially complete within 6-10 s. No significant increase in cell-associated ^{14}C was noted between 10 and 180 s; however, a second, much slower, linear phase of drug accumulation occurred for at least 5 h. The rate of ^{14}C accumulation increased progressively over a range of extracellular thiotepa concentrations (5 and 100 nmol/mL [5 and 10 µM]); however, K_m or V_{max} values could not be calculated due to loss of cell viability at concentrations between 200 and 500 nmol/mL (200 and 500 µM). L1210 cells incubated in 50 nmol/mL (50 µM) thiotepa for 3 or 5 h displayed a linear accumulation similar to that shown with the above experiments.

As with L1210 cells, the initial phase of cellular accumulation in human RBCs was essentially complete within 10 s of incubation. Human RBCs differed from L1210 cells in that they did not display a detectable slower second phase of accumulation, reflecting "obvious and important differences in cell structure between the two cell types" (Egorin et al., 1989). In contrast to L1210 cells, human RBCs are virtually devoid of mitochondria and RNA and have no nuclei; thus human RBCs lack nucleic acids for thiotepa to alkylate and have fewer types of enzymes that might convert thiotepa into a nonexchangeable form (Egorin et al., 1989).

Tepa was not observed in any cell extract that Egorin et al. (1989) studied by TLC. The findings were consistent with *in vivo* studies that implicate liver as the primary source of thiotepa metabolism (Egorin et al., 1984; cited by IARC, 1990; Egorin et al., 1989).

6.1.3 Metabolism

Thiotepa and its metabolites have been assigned Roman numerals in Table 6-1 for identification in Figure 6-1. Phase I metabolism of thiotepa has been studied by Ng and Waxman (1991) and Waxman et al. (1989; cited by Le Blanc and Waxman, 1989). The reactions of thiotepa and human glutathione have been studied with ^{31}P NMR by Dirven et al. (1995; cited by Dirven et al., 1996) and have been reviewed by Dirven et al. (1996).

In vitro rat liver microsomal and *in vivo* experiments have shown that oxidation of thiotepa (I) , catalyzed primarily by cytochrome P-450 IIB1 and, to lesser extents, by P-450 IIC11 and IIC6, yields the major metabolite tepa (II) (Waxman et al., 1989; cited by Le Blanc and Waxman, 1989; Ng and Waxman, 1991). See Table 6-1.

In aqueous solution at pH 7.4, thiotepa was found to be a very stable compound ($t_{1/2}$ = 3300 min); however, when *in vitro* incubations containing thiotepa and human glutathione were conducted at pH 7.4, the rate of metabolism increased substantially as determined by the rate of "disappearance" of thiotepa ($t_{1/2}$ = 282 min). Both mono- (III) and diglutathionyl thiotepa (IV) were identified as conjugates of thiotepa incubated with glutathione. The rate of formation of III was increased in incubations containing GSTA1-1 and P1-1, but not in incubations containing GSTM1a-1a and A2-2. The K_m for the formation of III in incubations including GSTA1-1 or P1-

1 was relatively high, with concentrations between 5-7 mM. Dirven et al. (1996) stated that this finding is not unusual for hydrophilic substrates like thiotepa. The rate of formation of IV was not increased in the presence of GSTA1-1, suggesting that only thiotepa is a substrate for GSTs, and that III is not. In the presence of GSTA1-1 and P1-1, the monoglutathionyl conjugates of tepa (II) were greatly enhanced.

Nonenzymatic formation of IV was not observed in the pH range 5.7-7.0 but was slightly increased at pH ~7.4-8.5 [~1-3 M]. The rate of formation of IV was increased in incubations including GSTA1-1 [~5-48 M] or P1-1 [~3-25 M] and thiotepa in the pH range 5.5-8.5. This suggests that nonenzymatic reaction of the aziridinium moieties with glutathione is greatly dependent on pH (Dirven et al., 1995; cited by Dirven et al., 1996).

6.2 Pharmacokinetics

The pharmacokinetics of thiotepa in humans, rodents, and primates have been reviewed by Lind and Ardiet (1993), IARC (1990), and Le Blanc and Waxman (1989) and are summarized below.

Summary: In humans, absorption of thiotepa after oral administration was erratic and incomplete, and was thought to be due to acid instability. A biexponential disappearance from plasma was observed following an i.v. bolus injection of thiotepa, with a second phase half-life reported as 73.7 min. Plasma clearance levels were reported to decline with increasing dose and metabolism of thiotepa to tepa is less efficient at the high dose. After intramuscular or i.v. administration of thiotepa, plasma clearance half-lives of 1.3-2.1 h were reported. Tepa was detected in the blood 5 min after an i.v. injection of thiotepa. Subsequently, the concentration of thiotepa in blood was lower than that of tepa. The ratio of thiotepa concentrations in cerebral ventricle fluid to plasma was approximately 1000 following intraventricular administration of thiotepa.

Similar to humans, a bioexponential decline in thiotepa concentration in plasma following an i.v. injection to mice was observed; however, mice displayed an earlier second phase $t_{1/2}$ as opposed to humans.

The total body clearance of thiotepa i.v. administered to monkeys was about 35 mL/min; equilibrium with plasma levels in lumbar and ventricular cerebrospinal fluid was obtained rapidly.

6.2.1 Pharmacokinetics in Humans

Absorption of thiotepa after oral administration was erratic and incomplete, and was thought to be due to acid instability (Mellet et al., 1962; cited by IARC, 1990). A biexponential disappearance from plasma was observed following an i.v. bolus injection of thiotepa (12 mg [63 μmol]/m^2), with a second phase half-life ($t_{1/2}$) reported as 73.7 min (Egorin et al., 1985; cited by IARC, 1990; Lind and Ardiet, 1993). At dose levels greater than 180 mg/m^2 (950 μmol/m^2) (Henner et al., 1987; cited by IARC, 1990), 25 mg/m^2 (132 μmol/m^2) (Heideman et al., 1989; cited by IARC, 1990), and 4.8 mg/kg (25 μmol/m^2) (Ackland et al., 1988; cited by IARC, 1990), plasma clearance levels were reported to decline with increasing dose. Ackland et al. (1988; cited by Lind and Ardiet, 1993) reported that the ratios of the area under the curve (AUC) for

thiotepa to the AUC for tepa was 13% as opposed to 33% in the study conducted by Cohen et al.(1986; cited by IARC, 1990; Lind and Ardiet, 1993), suggesting that metabolism of thiotepa to tepa is less efficient at the high dose. In contrast, Lazarus et al. (1987; cited by IARC, 1990) found that high doses (45-1215 mg/m^2 [240-6421 μmol/m^2]) did not produce dose-dependent kinetics.

After intramuscular or i.v. administration of thiotepa, plasma clearance half-lives of 1.3-2.1 h were reported in several studies (Heideman et al., 1989; Hagen et al., 1988; Henner et al., 1987; Hagen et al., 1987; Cohen et al., 1986; McDermott et al., 1985; all cited by IARC, 1990). The total body clearance of 12 mg/m^2 (63 μmol/m^2) thiotepa following i.v. injection was 186 ± 20 SD mL/min/m^2, the volume of the central compartment (not defined) was 0.25 ± 0.004 SD kg^{-1}, and the distribution volume at steady state was 0.71 ± 0.11 SD kg^{-1} (Cohen et al., 1986; cited by Lind and Ardiet, 1993).

Tepa was detected in the blood 5 min after an i.v. injection of thiotepa. Subsequently (120 min after injection), the concentration of thiotepa in blood was lower than that of tepa (Cohen et al., 1986; cited by IARC, 1990). Several studies have shown that within 8 h of injection, the urinary excretion of unchanged thiotepa is complete (Hagen et al., 1987; Cohen et al., 1986; Egorin et al., 1985; Hagen et al., 1985; all cited by IARC, 1990). In urine, during the first 8 hours after administration of thiotepa, tepa accounted for 4.2% and thiotepa accounted for 1.5% of the total dose administered; other alkylating metabolites were detected in the urine and corresponded to 23.5% of the administered dose (Cohen et al., 1986; cited by IARC, 1990; Lind and Ardiet, 1993).

Pediatric patients (condition not specified) administered thiotepa i.v. showed a cerebrospinal fluid-to-plasma ratio of 0.92 (Heideman et al., 1989; cited by IARC, 1990). The ratio of thiotepa concentrations in cerebral ventricle fluid to plasma was approximately 1000 following intraventricular administration of thiotepa (Strong et al., 1986 cited by IARC, 1990; Lind and Ardiet, 1993). A similar study conducted by Grochow et al. (1982; cited by IARC, 1990) showed that the ratio of thiotepa concentrations in cerebral ventricular fluid to plasma was approximately 200 (20% of ratio reported by Strong et al., 1986; cited by IARC,1990; Lind and Ardiet, 1993).

6.2.2 Pharmacokinetics in Rodents and Primates

6.2.2.1 Rodents

Similar to humans, a bioexponential decline in thiotepa concentration in plasma following an i.v. injection of 5 mg/kg bw (26 μmol/kg bw) to Swiss-Webster mice was observed (Egorin et al., 1984; cited by IARC, 1990); however, mice displayed an earlier second phase $t_{1/2}$ (9.62 min) as opposed to humans (73.7 min) (Egorin et al., 1985; cited by IARC, 1990; Lind and Ardiet, 1993).

6.2.2.2 Primates

The total body clearance of thiotepa i.v. administered to rhesus monkeys was about 35 mL/min; equilibrium with plasma levels in lumbar and ventricular cerebrospinal fluid was obtained rapidly (Strong et al., 1986; cited by IARC, 1990; Lind and Ardiet, 1993).

Lind and Ardiet (1993) stated that "As yet, there are no data correlating pharmacokinetics with toxicity and/or efficacy."

6.3 Modes of Action

Thiotepa and its major metabolite, tepa, are potent alkylating agents with strong mutagenic and clastogenic activity in *in vitro* and *in vivo* systems, including human (see section 5.0). This alkylating ability is the most likely explanation for its carcinogenicity in animals and humans.

6.4 Structure-Activity Relationships

Thiotepa is structurally related to other carcinogens containing three-ring heterocyclic moieties, e.g., propylene and ethylene oxides, diepoxybutane, diglycidyl resorcinol ether, and 4-vinyl-1-cyclohexene diepoxide (NTP, 1994) and aziridine (ethylenimine), 2-(1-aziridinyl)ethanol, and aziridinyl benzoquinone (IARC, 1975; IARC, 1987). The three-membered heterocyclic rings are easily opened to electrophilic species capable of alkylating DNA.

6.5 Cell Proliferation

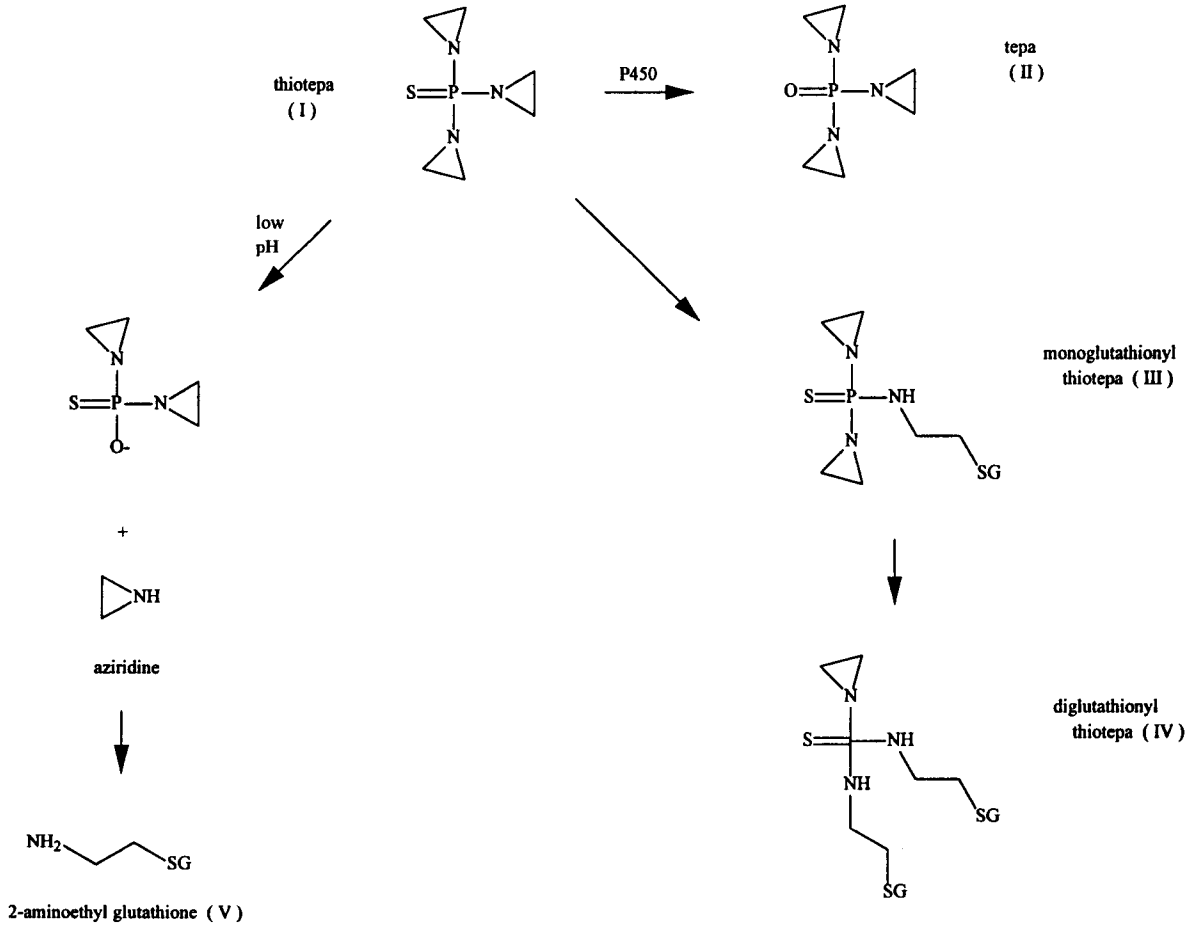
No studies were found that investigated whether thiotepa induces cell proliferation in experimental animals or in humans.

Table 6-1. Thiotepa Metabolite Identification

Metabolite[a]	Class of Reaction	Species	Reference
Tris(1-aziridinyl)-phosphine oxide; tepa (II)	Oxidation catalyzed primarily by cytochrome P-450 IIB1, and by P-450 IIC11 and IIC6 to a lesser extent	Sprague-Dawley rat	Boone et al. (1962; cited by IARC, 1990)
		dog	Mellet and Woods (1960; cited by IARC, 1990)
		rat, rabbit, dog	Craig et al. (1959; cited by IARC, 1990)
		human	Cohen et al. (1986; cited by IARC, 1990)
		rat, rat liver microsomes	Waxman et al. (1989; cited by Le Blanc and Waxman, 1989; Ng and Waxman, 1996).
Monoglutathionyl thiotepa (III)	Conjugation of I (GSTA1-1 and/or P1-1)	*In vitro* reaction with human glutathione incubated with I	Dirven et al. (1995; cited by Dirven et al., 1996).
Diglutathionyl thiotepa (IV)	Conjugation of I (GSTA1-1 and/or P1-1)	*In vitro* reaction with human glutathione incubated with I or III	Dirven et al. (1995; cited by Dirven et al., 1996).
2-Aminoethyl glutathione (V)	Conjugation via nonenzymatic reaction with glutathione at low pH	*In vitro* reaction with human glutathione	Dirven et al. (1995; cited by Dirven et al., 1996).
Inorganic phosphate	Oxidation	mouse	Craig et al. (1959; cited by IARC, 1990)

[a]Corresponds to the Roman numeral assigned to the parent compound or metabolite identified in Figure 6-1.

Figure 6-1. Reactions of Thiotepa with Glutathione (GSH)



Source: Dirven et al. (1995; cited by Dirven et al., 1996)

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR THIOTEPA

DESCRIPTION OF ONLINE SEARCHES FOR THIOTEPA (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 except for tepa [tris(aziridinyl)phosphine oxide], which is thiotepa's major metabolite.

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): 968 records were indexed by thiotepa's Chemical Abstracts Service Registry Number (CASRN) and 887 by the name thiotepa, giving a total of 1390 records indexed by one and/or the other. This number was reduced to 646 by selecting only records indexed by the MESH (Medical Subject Heading) term metabolism+all or by the MESH term neoplasms+all or selecting records that included the free text terms (question mark indicates truncation so that all forms of the word are included) carcinog? or mechanis? or pharmacokinetic? or toxicokinetic? or metab? Of these, 204 had been published after 1989. The titles of 237 records indexed by tepa's CASRN (545-55-1) were examined for duplicates with the thiotepa results. Records on chemosterilant activity for tepa in target organs were omitted unless genetic effects appeared to be covered. Approximately 160 of the records appeared to be of possible interest. After Dr. James Huff evaluated the combined thiotepa and tepa search results, approximately 40 publications were selected for retrieval.

CANCERLIT: The CANCERLIT strategy was the same as that for MEDLINE, described below. The total 1316 records was combined with the keywords to give 1106 records, which were further reduced to 557 published after 1988. When records containing the word "therapy" in their controlled vocabulary were eliminated, the total was reduced to 357 records. Of these, 17 appeared to be unique, and 12 were selected for acquisition.

EMIC/EMICBACK: Approximately 420 records were indexed by the CASRN.

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

EMBASE: The strategy used was identical to that used in MEDLINE, described below. After the 81 resulting records were examined, about 16 unique publications were selected for acquisition.

MEDLINE: In the entire database, a total of 1861 records was indexed by name (1861) and/or CASRN (707). These records were reduced to 1393 records by combining them with the truncated free text terms carcinogen? or mechanis? or toxicokinetic? or pharmacokinetic? or metaboli? or neoplas? or hyperplas? or metaplas? or foci? or tumor? or tumour? Among these records, 326 of the records represented papers published after 1988. These were reduced to 211 records by eliminating records that included the word "therapy" in their controlled vocabulary. After examination of these records and elimination of duplication with TOXLINE records, an additional 13 records were selected for acquisition of the publications.

TOXLIT: The strategy used in this database was the same as that used in MEDLINE. After the 96 resulting records were examined, 18 unique publications 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