THIOCARBONYL COMPOUNDS

CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

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5.2.2.8.1 Introduction.

Interest in the pharmacological and toxicological studies of this chemical class arose in the early 1940's when the goitrogenic properties of allylthiourea and other related compounds were first noted. In 1941, Kennedy and Purves (1) and Griesbach (2) found that rats fed a diet containing brassica seeds developed goiter. Suspecting that a thiourea derivative in the brassica seeds might be responsible for the goitrogenic effect, Kennedy (3) administered allylthiourea to rats and noted the induction of goiter in the animals. Other thiourea related compounds were later shown to possess various degrees of goitrogenic activity (4-7). These goitrogenic agents produce thyroid hypertrophy and hyperplasia by inhibiting the synthesis of thyroid hormones, thereby triggering the secretion of thyroid stimulating hormone (TSH) from the pituitary gland. A large volume of literature has accumulated describing the effects of these compounds on the endocrine function of the thyroid gland. Because of their antithyroid activity, a number of them including thiouracil (TU), methylthiouracil (MTU) and propylthiouracil (PTU) were, at one time, considered as drugs of choice for the treatment of hyperthyroidism.

Moreover, several thiocarbonyl compounds acquired economic importance because of their extensive applications in the industry and as pesticides (see Section 5.2.2.8.5). For instance, in 1945 thiourea and thioacetamide (TAA) were found to be effective fungicides and were used in this capacity in the prevention of orange decay (8). It was the presence of these chemicals in the juice of treated oranges that raised great concern of the possible health hazards to humans. Extenxive toxicological studies have since been carried

out on these compounds. Ethylenethiourea (ETU) has been shown to be one of the major degradation products of ethylene bis-dithiocarbamates, an important and widely used class of fungicides for the treatment of diseases of a large variety of agricultural crops (cited in ref. 9; see also Section 5.2.1.6). It has been established in animal bioassays that ETU is carcinogenic, mutagenic and teratogenic. Because of the potential hazard that ETU represents to human health, a Rebuttable Presumption Against Registration (better known under the acronym RPAR) of all pesticide products containing ethylene bis-dithiocarbamate was issued by the U.S. Environmental Protection Agency in 1977 (10). The National Institute for Occupational Safety and Health (NIOSH) also recommended to minimize occupational exposure to ETU and to handle the compound in the workplace as if it were a human carcinogen and teratogen (11).

Over half a century ago, Wegelin (12) noted the existence of a link between endemic goiter and malignant tumor of the thyroid gland. He believed that benign goitrous hyperplasia predispose to the development of malignant thyroid neoplasms both in animals and in humans. His view has been substantiated by histopathological studies which reveal that most thyroid cancers of humans are derived from goitrous glands. Since spontaneous thyroid tumors rarely occur in laboratory rodents, goitrogen-induced thyroid tumors in rats and mice have become a valuable model for studies on hormone-dependent tumors as well as the role of goitrogenic agents in carcinogenesis.

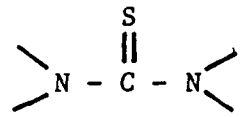
The first study on experimental tumor induction by a thiocarbonyl goitrogen dates back to 1944 when Bielschowsky (13) demonstrated the emergence of benign and malignant tumors in the thyroid gland in rats by the concurrent feeding of allylthiourea and 2-acetylaminofluorence (2-AAF). Subsequent studies (14) showed that allylthiourea alone also induces adenomata in the rat thyroid, although the incidence is lower than that produced by the combined

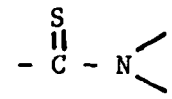
action of allylthiourea and 2-AAF. Since then many investigations have been carried out on thyroid tumor induction in rodents by antithyroid drugs. Several thiourea related compounds including trimethylthiourea, diethylthiourea, dicyclohexylthiourea, dithiobiurea and phenylthiourea have been tested for carcinogenicity by the U.S. National Cancer Institute, because of occupational exposure to these compounds in their industrial use. Ethionamide, a synthetic antituberculotic drug structurally related to thioacetamide, was also tested by the U.S. National Cancer Institute because it is often used clinically for extended periods of time.

5.2.2.8.2 Physical and Chemical Properties & Biological Effects.

5.2.2.8.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

Table I presents the structural formula, molecular weight, solubility and other physical properties of thiocarbonyl compounds which have been bioassayed for carcinogenicity. These compounds include thiourea and its aliphatic and heterocyclic derivatives, which all contain a thioureylene group:



In thioacetamide and ethionamide, one of the nitrogen atoms is replaced by a methyl and a 2-ethylpyridine group, respectively. In these compounds only the thioamide group  is common with other compounds of the class.

Thiourea reacts with various metals under neutral conditions and forms adducts or complexes. Prolonged heating of thiourea at 170°C yields ammonium thiocyanate (15). Thioacetamide is relatively stable in neutral aqueous

Table I. Chemical and Physical Properties of Thiocarbonyl Compounds[a]

Compound	Structure	M.W.	Physical properties	Solubility
Thiourea	$H_2N - \overset{\overset{S}{\|\|}}{C} - NH_2$	76	White, glossy crystal; bitter taste; sp. gr. 1.406; m.p. 176-182°C; sublimes at 150-160°C under vacuum	Soluble in cold water, ammonium thiocyanate and ethanol; sparingly soluble in ether
Allyl-thiourea	$CH_2 = CHCH_2 - NH - \overset{\overset{S}{\|\|}}{C} - NH_2$	116	Colorless monoclinic crystal; garlic odor; m.p. 74°C	Soluble in water
Trimethyl-thiourea	$(CH_3)_2N - \overset{\overset{S}{\|\|}}{C} - NHCH_3$	118	Prism; m.p. 87-88°C	Soluble in water, ethanol, and trichloromethane
N,N'-Diethyl-thiourea	$C_2H_5NH - \overset{\overset{S}{\|\|}}{C} - NHC_2H_5$	132	Crystal; m.p. 68-71°C	Slightly soluble in water; soluble in methanol, ethanol, ether, acetone, benzene, and ethyl acetate
Phenyl-thiourea	$C_6H_5 - NH - \overset{\overset{S}{\|\|}}{C} - NH_2$	152	Needle-like crystal; bitter taste; m.p. 152°C	Soluble in water and ethanol
N,N'-Dicyclo-hexylthio-urea	$C_6H_{11} - NH - \overset{\overset{S}{\|\|}}{C} - NH - C_6H_{11}$	240	m.p. 182°C	--
2,5-Dithio-biurea	$H_2N - \overset{\overset{S}{\|\|}}{C} - NH - NH - \overset{\overset{S}{\|\|}}{C} - NH_2$	118	m.p. 214°C	--

Table I. Chemical and Physical Properties of Thiocarbonyl Compounds[a] (continued)

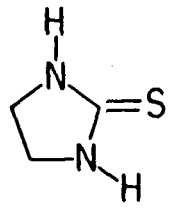
Compound	Structure	M.W.	Physical properties	Solubility
Ethylene thiourea (ETU)	(see Footnote b)	102	White to green crystal; faint amine odor; m.p. 199-204°C	Soluble in hot water; slightly soluble in cold water, methanol, ethanol, acetic acid, and naphtha
Thiouracil (TU)	(see Footnote b)	128	White powder or minute crystal; bitter taste; melts with decomposition at about 340°C; combustible	Readily soluble in alkaline solutions; very slightly soluble in water, ethanol, and ether
Methyl-thiouracil (MTU)	(see Footnote b)	142	White crystal; bitter taste; sublimes readily; decomposes at about 330°C	Soluble in aqueous solutions of ammonia and alkali hydroxides; slightly soluble in ethanol and acetone; very slightly soluble in cold water and ether
Propyl-thiouracil (PTU)	(see Footnote b)	170	White powdery crystalline substance; starch-like in appearance and to touch; bitter taste; sensitive to light; m.p. 218-221°C	Soluble in ammonia and alkali hydroxides; sparingly soluble in ethanol; very slightly soluble in water
Thioacetamide (TAA)	$CH_3 - \overset{\overset{S}{\|}}{C} - NH_2$	75	Colorless leaflet; slight odor of mercaptans; m.p. 109-114°C	Soluble in water and ethanol; slightly soluble in water

Table I. Chemical and Physical Properties of Thiocarbonyl Compounds[a] (continued)

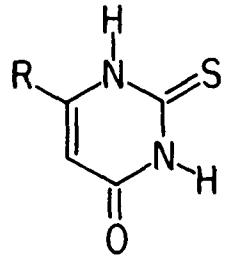
Compound	Structure	M.W.	Physical properties	Solubility
Ethionamide	(see Footnote b)	166	Minute yellow crystal; m.p. 164-166°C	Soluble in hot acetone, dichloroethane and pyridine; slightly soluble in methanol, ethanol, propylene glycol; very sparingly soluble in water

[a]Compiled from: Hawley, G.G., (ed)., "The Condensed Chemical Dictionary," 9th ed. Van Nostrand, New York, 1977; Verschueren, K., "Handbook of Environmental Data on Organic Chemical," Van Nostrand, New York, 1977; Gleason, M.N., Gosselin, R.E., Hodge, H.C. and Smith, R.P.: "Clinical Toxicology of Commercial Products," 3rd ed. Williams & Wilkins Baltimore, 1969; Sax, N.I., "Dangerous Properties of Industrial Materials," 5th ed. Van Nostrand, New York, 1979; Merc Index, 9th ed., Merck & Co., Rahway, New Jersey, 1976.

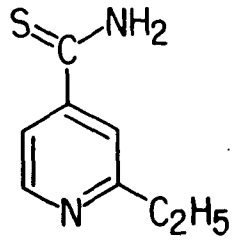
[b]Formulas to Table above:



Ethylene thiourea (ETU)



Thiouracil (R=H; TU)
MTU(R=CH_3)
PTU(R=C_3H_7)



Ethionamide

solutions. However, if heated at acidic or alkaline pH, it undergoes hydrolysis and releases hydrogen sulfide or sulfide ion (16).

5.2.2.8.2.2 Biological Effects Other Than Carcinogenic

Goitrogenicity. The goitrogenic effect of thiourea and related compounds in rats has been known since the report by Kennedy (3) and the extensive investigations of Astwood and coworkers (6, 7). These agents act directly on the thyroid gland to inhibit the synthesis of thyroxine through the inhibition of a peroxidase which mediates the conversion of iodide ion to iodine radical. The resulting low level of thyroid hormone in the circulation triggers an increased secretion (from pituitary glands) of thyroid stimulating hormone (TSH) which acts on the thyroid epithelium to cause hyperthophy and hyperplasia. Astwood et al. (7) determined the antithyroid activities of a large number of compounds in rats. The decrease in thyroid iodine concentration and increase of weight of the thyroid gland were used as parameters to compare the activities of the compounds with TU, which was chosen as a standard of reference and assigned an arbitrary activity of 1. The relative antithyroid activities of some thiocarbonyls were found to be: MTU, 1.00; PTU, 11.00; thiourea, 0.12; trimethylthiourea, 0.10; diethylthiourea, 0.40; dithiobiurea, <0.01; thioacetamide, <0.01. The most active compounds are derivatives of TU. Lindsay et al. (17) have also demonstrated that only thiopyrimidines, but not thiopurines, are goitrogenic in rodents. Substitution by alkyl or aryl groups at position 6 of thiouracil leads to enhanced activity; the highest activity is with a propyl group. Thyroid glands weighing over 100 times normal weight have been observed in rats administered PTU (18). A further increase in the length of the side chain results in decreased activity. Thiourea exhibits only 1/8 to 1/10 activity of TU. Substitution by

methyl groups of up to 3 hydrogens in thiourea does not significantly change the goitrogenic activity. However, when the substituents on the thiourea molecule are too large, activity is generally reduced or lost (7).

In humans, thiourea is as goitrogenic as thiouracil. Among the various 6-substituted thiouracils studied, only the methyl derivative was found more active than the parent compound. Substitution with polar groups at position 6 greatly reduces the activity (19).

Toxicity. Most early toxicity screening studies on this chemical class were performed in view of its use for the treatment of thyrotoxicosis and as rodenticides. The toxicity data show that thioureas containing a benzene ring are much more toxic than other thiourea derivatives (20, 21). For example, phenylthiourea, administered either orally or by intraperitoneal injection, exhibits high acute toxicity in rats, mice and rabbits. Most other thiourea derivatives are relatively non-toxic in rats or mice; few have LD_{50} value lower than 100 mg/kg (Table II).

The manifestations of acute poisoning by thiourea and by its derivatives are massive pulmonary edema, pleural effusion, and tracheobronchial inflammation (21, 28). Toxic doses of thiourea and several of its monosubstituted analogs, including allylthiourea and phenylthiourea, also cause hyperglycemia and depletion of liver glycogen in rats (24, 29). The disturbances in carbohydrate metabolism are believed to be secondary to the pulmonary effects, which are the direct cause of death in thiourea toxicity (29).

The side effects of the therapeutic use of TU and its analogs are well established. Patients treated with TU, MTU, or PTU may develop skin rash, headache, fever, edema, hepatic necrosis, aplastic anemia and agranulocytosis (19, 30, 31). Allergic reactions, gastrointestinal disturbances and toxic

Table II. Acute Toxicity of Thiocarbonyl Compounds

Compound	Species & Route	LD_{50} (mg/kg)	Reference
Thiourea	Rat, oral	125	(22)
	i.p.	436	(23)
	Mouse, oral	8,500	(22)
	i.p.	100	(22)
Allylthiourea	Rat, oral	200	(21)
	i.p.	500	(24)
Trimethylthiourea	Rat, oral	316	(22)
	Mouse, oral	215	(22)
Diethylthiourea	Rat, oral	316	(22)
	Mouse, oral	681	(22)
	i.p.	500	(22)
Phenylthiourea	Rat, oral	3	(21)
	i.p.	5	(24)
	Mouse, oral	10	(22)
	i.p.	25	(22)
	Rabbit, oral	40	(25)
Dicyclohexylthiurea	Rat, oral	1,500	(21)
Dithiobiurea	Mouse, i.p.	50	(22)
Ethylenethiourea (ETU)	Rat, oral	1,832	(26)
Thiouracil (TU)	Rat, oral	2,000	(21)
Methylthiouracil (MTU)	Mouse, i.p.	200	(22)
Thioacetamide (TAA)	Rat, oral	500	(21)
	Mouse, i.p.	300	(22)
Ethionamide	Mouse, oral	1,000	(27)
	i.p.	1,350	(22)

hepatitis have been associated with the use of ethionamide in the treatment of tuberculosis (32).

Thioacetamide is a well-known liver necrogenic agent. In rats, toxic doses of thioacetamide rapidly cause hepatic cell injury which is followed by centrolobular necrosis (16, 33-35). The most striking early effects in the hepatic cells are the doubling of nuclear volume, increased nucleolar and cell volume, mitochondrial swelling, distention of the endoplasmic reticulum cisternae, detachment of the ribosomes and polysomes from the rough endoplasmic reticulum and loss of cytoplasmic azurophilia (34, 36, 37). Chronic administration to rats produced cirrhosis of the liver characterized by irregular fibrosis, hyperplasia of the bile ducts, fatty infiltration and nodular regeneration of the parenchymal cells (33, 38).

Mutagenicity. Both thiourea and thioacetamide were selected for mutagenesis assay by the U.S. National Cancer Institute for determining the extent of correlation between carcinogenesis and mutagenesis in several standardized assay systems (39). In agreement with earlier results of McCann et al. (40), the National Cancer Institute results (41, 42) show that both thiourea and thioacetamide are not mutagenic in various strains of Salmonella typhimurium, assayed in the presence and absence of the S-9 mix. However, in the host-mediated assay, both thiourea and thioacetamide at doses of 125 mg/kg produced significant increases in the mutation frequencies observed with S. typhimurium TA1530 and TA1538, indicating that the two compounds are probably weak mutagens (43). Thioacetamide, but not thiourea, is mutagenic in Saccharomyces cerevisiae D3 (44) and Drosophila melanogaster (45). Both compounds displayed weak mutagenicity in S. cerevisiae D6 (46) and a forward mutation system, gal^r, in S. typhimurium (47). Recently, Yamaguchi (48) has also demonstrated moderate mutagenicity of thiourea in S. typhimurium TA100, a tester strain

previously shown to have no response to the compound (40, 41). Thioacetamide, on the other hand, did not cause detectable unscheduled DNA synthesis in rat liver (49) or in a primary culture of rat hepatocytes (50).

In a study on the validity of various carcinogen screening techniques, 182 compounds were examined by several short-term assays in Japan between 1973 and 1978 (51). Both thiourea and thioacetamide exhibited negative results in strains TA98 and TA100 of S. typhimurium, in hamster lung fibroblast cells and in rat bone-marrow cells (the latter assays tested for chromosomal aberrations). Thioacetamide, but not thiourea, was mutagenic in Bacillus subtilis; thiourea, but not thioacetamide, however, caused mutations in silk worms.

Extensive research on the mutagenic effects of ethylenethiourea (ETU) has also been conducted over the past few years, mainly because of its association with ethylene bis-dithiocarbamate fungicides. The weak mutagenicity of ETU was demonstrated in several microbial systems: S. typhimurium TA98, TA100, TA1538 (52), TA1535 (52, 53), TA1530 (54, 55), his G-46 (56), and S. cerevisiae D6 (46). Cytogenetic analysis also indicates the weak mutagenicity of ETU. A dose-dependent increase in the frequency of chromosomal aberrations was observed in bone marrow cells of mice administered ETU at single or repeated doses (57). Moreover, Herichova (58) found chromosomal aberrations and induction of micronuclei in the root tips of Vicia faba treated with ETU.

A number of laboratories were unable to confirm, however, some of the above findings and failed to detect mutagenic activity of ETU in several other assays systems. Saffiotti et al. (59) reported that ETU produced no revertants in TA98 and TA100, the two S. typhimurium tester strains which respond respectively to frame-shift and to base substitution mutagens. Also, Shirasu et al. (60) observed no mutagenic activity of ETU in the S. typhimurium

TA1536-8 series, in Escherichia coli WP2 and in Bacillus subtilis. Furthermore, negative results were obtained in mouse dominant lethal tests (54, 57, 61, 62), as well as in cytogenetic studies on cultured cells (62-64) and in whole animals (54, 55, 57, 62, 63, 65). The frequencies of sex-linked recessive lethals and dominant lethals in Drosophila treated with ETU were also not significantly higher than those of the controls (66).

Studies on the mutagenicity of other thiocarbonyls are more limited. Allylthiourea and phenylthiourea displayed moderate mutagenic activities when tested on S. typhimurium TA100 without metabolic activation (48). Assays for mutagenicity based on the reversion from streptomycin dependence to independence in strain Sd-4-73 of E. coli showed that neither TU, MTU, nor PTU were mutagenic (67). The incidence of thyroid cells containing chromosomal aberrations was also not increased by the administration of PTU to rats for 15 weeks (68). However, PTU was reported to induce "petite" mutations in S. cerevisiae D6 (46). Testing for mutagenic effects of ethionamide by using S. typhimurium TA98, TA100, B. subtilis, Chinese hamster lung fibroblasts (testing for chromosomal aberrations), human embryo fibroblasts (testing for chromosomal aberrations and sister chromatid exchanges), bone marrow cells (testing for chromosomal aberrations), and silk worms, Kawachi et al. (51) obtained positive results only with Chinese hamster lung fibroblasts.

Teratogenicity. Since the advent of thiourea and TU, sporadic goiter and hypothyroidism in fetuses and newborn infants due to the use of these drugs and their derivatives (e.g., PTU and MTU) by mothers during pregnancy have been noted (69-76). The effects on infants are transient and usually disappear shortly after birth; however, mental development may be subsequently retarded in some congenitally hypothyroid children (70, 77). Administration of thiourea, TU and their derivatives to pregnant rats has been found to cause

thyroid hyperplasia and abnormal body development in the offspring (78-83). Similar effects were observed in weanling rats whose mothers had been treated with thiourea or TU during the period of lactation (82, 84). Various investigators have shown that both thiourea and TU pass through the placenta to the fetus (79, 85-91) and are secreted in the milk of humans (92) and animals (85, 90). Presumably, transient fetal or neonatal hypothyroidism results from the inhibition of thyroxine biosynthesis in the fetus by the drugs, which then leads to increased production of fetal thyroid-stimulating hormone (TSH) and, thus, to thyroid hyperplasia. Recently, it has also been suggested that the effects might be due to the placental transfer of the thyroid-stimulating immunoglobulin from mother to fetus (75, 76).

Teratogenicity studies in the last decade, carried out principally by Khera and his associates, have established that ethylenethiourea (ETU) is a potent teratogen in the rat (26, 93-98). A wide spectrum of anomalies in the central nervous, urogenital and skeletal systems as well as in other organs have been demonstrated in the offspring of rats administered sublethal doses of ETU during pregnancy. Among the teratogenic effects observed are hydrocephalus, microphthalmia, meningoencephalocele, meningorrhagia, meningorrhea, obliterated neural canal, cleft palate, kyphosis, kinky tail and various defects of the digits. Studies by administration of single oral doses of ETU to rats at different gestation stages have revealed that the type of anomalies and organs affected varies with the treatment stages and coincides with the onset of organogenesis (94, 98). Ethylenethiourea is one of the most potent antithyroid agents known (99). Its teratogenic effects in rats are believed to be due neither to direct action on the affected organs (100) nor to alteration of the maternal thyroid activity, but are caused by direct effect on the fetal thyroid function (26, 96). A study with various chemicals structurally

similar to ETU established that the 2-mercaptoimidazolidine structure was essential for producing the teratogenic effects (101).

Ethylenethiourea has also been reported to be teratogenic in hamsters (102, 103). However, no prominent evidence was found for teratogenicity of ETU in rabbits (93), mice (104, 105) or cats (106). Results from pharmacokinetic studies in mice by Ruddick et al. (104, 105) led to the suggestion that a faster clearance of ETU from the fetal tissues and a more rapid metabolism of ETU to non-teratogenic compounds might be the cause for the lack of teratogenicity of ETU in this species.

Skeletal malformations have been observed in the offspring of rats administered the anti-mycobacterial tuberculostatic drug, ethionamide, on days 6 to 14 of pregnancy (cited in ref. 107). The drug was not found, however, to exert any significant adverse effect on the development of the human fetus (108, 109).

5.2.2.8.3 Carcinogenicity.

5.2.2.8.3.1 OVERVIEW.

Spontaneous tumors of the thyroid in rodents are rare (110, 111). Experimental thyroid tumors have been induced in rats and mice by low-iodine diet, radioactive iodine, chemicals, ionizing radiation, or by partial thyroidectomy (reviewed in 112). Thiourea and related compounds, which are goitrogenic, also induce thyroid adenomas and carcinomas and/or tumors of other organs after prolonged administration to laboratory animals (Table III). Hence, thiourea-type goitrogens represent valuable research tools for the biochemistry and physiology of the thyroid gland, as well as for the

Table III. Carcinogenicity of Thiocarbonyl Compounds

Compound	Species and Strain	Route	Principal Organs Affected	Reference
Thiourea	Rat, Wistar, albino	Oral	Thyroid gland	(113, 114)
	Rat, albino	Oral	Liver	(115, 116)
	Rat, Hebrew University strain	i.p. and oral	Face, eyelid, ear, nose	(117, 118)
	Mouse, A, C57, I, hybrids, or C3H	oral	None	(119-122)
	Mouse, C3H	oral	Thyroid gland[a]	(123)
	Mouse, AKR	oral	Skull	(124)
	Fish, rainbow trout	oral	Liver	(125)
Allylthiourea	Rat, Wistar	oral	Thyroid gland	(14, 126)
N,N'-Diethylthiourea	Rat, Fischer 344	oral	Thyroid gland	(127)
	Mouse, B6C3F1	oral	None	(127)
Trimethylthiourea[b]	Rat, Fischer 344	oral	Thyroid gland	(128)
	Mouse, B6C3F1	oral	None	(128)
Phenylthiourea	Rat, Fischer 344	oral	None	(129)
	Mouse, B6C3F1	oral	None	(129)
N,N'-Dicyclohexyl-thiourea	Rat, Fischer 344	oral	None	(130)
	Mouse, B6C3F1	oral	None	(130)
Dithiobiurea	Rat, Fischer 344	oral	None	(131)
	Mouse, B6C3F1	oral	None[c]	(131)

Table III. Carcinogenicity of Thiocarbonyl Compounds
(continued)

Compound	Species and Strain	Route	Principal Organs Affected	Reference
Ethylenethiourea (ETU)	Rat, Charles River CD	oral	Thyroid gland	(132-135)
	Rat, --	oral	Thyroid gland	(136)
	Mouse, $B6C3F_1$ or $B6AKF_1$	oral	Liver, hematopoietic system	(137)
	Hamster, --	oral	None	(136)
Thiouracil (TU)	Rat, Stanford, Sherman, or Sprague-Dawley	oral	Thyroid gland	(138-141, cited in ref. 12)
	Mouse, C3H or (C57 X CBA)F1	oral	Liver	(142-144)
	Mouse, TM	oral	None	(143)
Methylthiouracil (MTU)	Rat, Wistar, Lister, albino, or Long-Evans	oral	Thyroid gland	(145-154)
	Rat, albino	oral	Kidney	(155)
	Mouse, $C3H^d$	oral	Thyroid, liver	(156)
	Mouse, NZO/Bl or $C57^d$	oral	Thyroid gland	(157, 158)
	Mouse, dd	oral	Pituitary gland	(159)
	Hamster, Syrian	oral	Thyroid gland	(160)
	Hamster, --	oral	Thyroid gland	(161)

Table III. Carcinogenicity of Thiocarbonyl Compounds (continued)

Compound	Species and Strain	Route	Principal Organs Affected	Reference
Propylthiouracil (PTU)	Rat, Wistar, albino, Long-Evans, or unspecified	oral	Thyroid gland	(162-168)
	Mouse, A	oral	Thyroid and pituitary glands	(169)
	Mouse, C57Bl	oral	Pituitary gland	(170)
	Hamster, Syrian golden	oral	Thyroid gland	(171)
	Guinea pig, --	oral	Thyroid gland	(172)
Thioacetamide (TAA)	Rat, albino or Wistar	oral	Liver, bile duct	(116, 173, 179)
	Mouse, Swiss	oral	Liver	(180)
	Hamster, --	oral	None	(181)
Ethionamide	Rat, Fischer 344	oral	None	(182)
	Mouse, B6C3F1	oral	None	(182)
	Mouse, BALB/c/Cb/Se	oral	Thyroid gland	(cited in ref. 183)

[a]One thyroid adenoma in 1 of 25 C3H male mice that have been castrated.

[b]Administered as a mixture of 85% trimethylthiourea and 15% dimethylthiourea.

[c]There was some suggestive evidence that dithiobiurea may induce hepatocellular carcinomas in female mice.

[d]Kept on low-iodine diet.

elucidation of the mechanism of thyroid gland carcinogenesis. Since humans are exposed to many of these goitrogens, as pesticide residues or drugs for the treatment of thyrotoxicosis, their carcinogenicity must be adequately tested and their potential hazard to humans assessed. Over the past decades, a large volume of literature on experimental thyroid tumorigenesis has accumulated. Recently, bioassays of several thiourea related compounds for possible carcinogenicity have been conducted in rats and mice by the U.S. National Cancer Institute. Table III summarizes some of the carcinogenicity studies of thiourea and its analogs, discussed in some detail later in this section.

The development, morphology, histology and metabolism of thyroid gland tumors have been the subject of several reviews and monographs (112, 155, 183-187). In both rats and mice, different agents induce a histological variety of experimental thyroid tumors. Tumor development requires a long induction period and is often preceded by a hyperplastic stage. Following thyroid hyperplasia, local areas of proliferating altered cells develop; it is in these areas that the adenomas and/or carcinomas emerge. It is difficult to differentiate morphologically between hyperplastic nodules and benign and malignant forms of thyroid neoplasm. In the hyperplastic state, both the follicular and parafollicular epithelial cells undergo great variations of hyperplastic modification. In most cases, these cells become cylindrical with basophilic cytoplasm and a pale vesicular nucleus. Dalton et al. (188) distinguished two types of hyperplastic modifications: "Type I" represents cells which are larger in size than normal cells; "Type II" refers to cells that are similar in size to the normal surrounding cells but have more basophilic nuclei. In the adenomas, both cytoplasmic and nuclear changes are evident and the cellular distribution is more irregular than in hyperplasia (158). Induced thyroid adenomas and carcinomas are usually papillary or

follicular. Transplantability of these tumors is generally low, which presumably contributes to their low grade of malignancy. However, epidermoid carcinomas, which are more malignant than the papillary or follicular tumors, have also been observed (183). Thyroid tumors induced by thiourea goitrogens appear to be more easily transplanted in mice than in rats (112).

5.2.2.8.3.2 THIOUREA AND RELATED COMPOUNDS.

Thiourea. In 1945, Griesbach et al. (189) reported the induction of thyroid adenomas in rats by the long-term ingestion of brassica seeds, which are goitrogenic, in the diet. This observation stimulated a number of investigations on the carcinogenicity of thiourea and several goitrogenic thiouracils. As was expected from the results of the brassica seed experiment, Purves and Griesbach (113) observed thyroid tumors in most of the male Wistar rats receiving 0.25% thiourea in the drinking water for a period of nearly two years. Tumors in 2 of the 30 tumor-bearing animals were considered to be malignant; the malignant tumors metastasised to the lung and spread invasively into the blood vessels. This initial study was subsequently extended to include rats of a local (Norwegian) albino strain of both sexes (114). The results indicate that there are no significant sex- or strain-dependence in susceptibility of thyroid tumorigenesis. After a year or more, thyroid adenomas were found in 22 of 25 rats (88%) given 0.25% thiourea in the drinking water. Of 13 animals that survived more than 20 months, 7 had thyroid tumors which showed criteria of malignancy. In addition, 3 thyroid tumors resembling the "foetal adenoma" of human pathology were encountered. The observation that the thyroid was the only organ in which neoplasia developed led Purves and Griesbach (114) to the conclusion that the thyroid tumors induced are not the result of a direct carcinogenic action of thiourea but are the consequences of prolonged stimulation by thyrotropic hormone.

In contrast to the above findings, however, a number of reports have described the induction of tumors by thiourea in other organs. Fitzhugh and associates (115, 116) showed that thiourea, administered orally at dose levels of 0.1, 0.05, 0.025 and 0.01% in the diet for about 2 years, induced hepatic cell adenomas, without liver cirrhosis, in 14 of 29 albino rats. Levels of 1.0, 0.5 and 0.25% produced marked hyperplasia of the thyroid; however, no carcinogenic effect of thiourea was seen in these groups due to early death of all animals. None of the 18 controls surviving the 2-year feeding period developed tumors. Rosin and Rechmilewitz (117) observed malignant tumors of the face in 5 of 6 random-bred (Hebrew University strain) rats treated with thiourea for over 1 year. The treatment consisted of weekly intraperitoneal injections of 10% aqueous solution of thiourea, in doses of 3.0, 4.0 and 4.0 ml on 3 consecutive days, for a period of 6 months, followed by continued administration of 0.2% thiourea in the drinking water. Subsequent studies (118) confirmed the above findings and, in addition, observed tumors on the eyelids and near the ear ducts in 10 of 12 Hebrew University strain rats surviving the same treatments for over 1 year. Eighteen out of 19 rats which received 0.2% thiourea in the drinking water for the entire experimental period also developed neoplastic lesions after 26 months: 1 had a myxomatous tumor on the nose and 17 had epidermoid carcinomas near the ears and the orbit (118).

The chronic effects of thiourea on the microscopic structure of the thyroid in strains A, I, C57, and hybrid mice were investigated by Gorbman (119, 120). Administration of 0.2% thiourea in the diet induced hyperplasia and follicular cysts in the thyroid after 200-300 days of treatment. In 7 of the 31 strain A mice so treated for more than 500 days, "hyperplastic thyroid tissue metastases" in the lung were also noted. However, neither the thyroid

changes nor the pulmonary metastases in these mice were considered by the author to be of neoplastic nature, since all the lesions regressed after the resumption of a normal diet. These findings were confirmed by Dalton et al. (121, 122); moreover, these authors found no histological evidence for neoplasms in the thyroid of C3H mice treated with 0.25% thiourea in the diet for up to 63 weeks, despite pulmonary metastasis of thyroid tissue. Casas and Koppisch (123), on the other hand, observed a thyroid adenoma in 1 of 25 C3H mice that had been castrated, and received thiourea at the dose level of 0.3% in the diet for 7 months. Recently, a significantly increased incidence of intracranial bone tumor was found in AKR mice treated with a high dose of thiourea (5g/kg) in the diet (124). Thiourea has also been shown to induce hepatomas in rainbow trout (125).

Allylthiourea. Whereas 2-acetylaminofluorene did not affect the normal thyroid tissue, combined treatment of 2-acetylaminofluorene and allylthiourea induce benign and malignant tumors of the thyroid in rats (13, 145). Subsequent studies showed that thyroid neoplasms could be induced in rats given allylthiourea alone, although the tumorigenic effect was not as pronounced as with 2-acetylaminofluorene and allylthiourea combined. In one experiment, 5 young female Wistar rats were given 8 mg allylthiourea daily in the diet. After 9-14 weeks of treatment, 2 of the rats developed a single adenoma of the thyroid. In another study, a diet containing allylthiourea was fed to 5 male and 5 female Wistar rats born to and nursed by a mother bearing mammary tumors induced by 2-acetylaminofluorene. Single adenomas were found in 2 of the 5 males after 25 weeks and 3 of the 5 females after 19 weeks of stimulation by the goitrogen (14).

Ethylenethiourea (ETU). Data on ETU-induced thyroid carcinogenesis establish that the compound is clearly carcinogenic in both rats and mice.

A 1972 preliminary communication by Ulland and associates (132) reported the dose-related induction of thyroid tumors in Charles River CD rats administered two doses (175 and 350 ppm) of ETU in the diet for 18 months and observed for an additional 6 months. At the low dose level, 3 males and 3 females in groups of 26 animals developed thyroid carcinomas; at the high dose level, 17 males and 8 females had thyroid cancers. In addition, hyperplastic goiter and thyroid solid-cell adenoma occurred in a few other animals at both dose levels. The final results of this study have recently been published by Weisburger et al. (133). The incidences of follicular cell carcinoma of the thyroid were: 58% in high-dose males, 23% in high-dose females, and 8% in low-dose groups of either sex. In addition, 3 papillary carcinomas of the thyroid and 4 hyperplastic nodules of the liver were seen. No such neoplastic lesions were detected in matched or pooled controls.

Similarly, Graham et al. (134, 135) reported that there was a significant dose-related increase in the thyroid carcinoma incidence in Charles River rats administered ETU in the diet for 1 or 2 years. After 1-year administration of ETU , 60% of the rats at the dietary dose of 500 ppm developed thyroid carcinomas compared to 0% in the controls (134); in the 2-year study, 23% and 89% of the rats receiving levels of 250 and 500 ppm, respectively, had thyroid carcinomas or adenocarcinomas compared to 3% in the controls (135). Actually, the carcinogenicity of ETU toward the thyroid was already detectable at the 125 ppm dietary level; there was no detectable tumorigenicity at the 25 and 5 ppm levels.

In a life-time carcinogenesis study in rats and hamsters of unspecified strains, ETU was given at doses of 0, 5, 17, 60 and 200 mg/kg in the diet (136). The compound was found to be carcinogenic for male and female rats at 60 mg/kg level and 200 mg/kg level, respectively. Again, the target tissue

affected was the thyroid. No tumorigenic effect was observed in hamsters even at the highest dose level.

Innes et al. (137) have investigated the carcinogenicity of ETU in mice of 2 hybrid strains (C57Bl/6 X C3H/Anf; C57Bl/6 X AKR). At 7 days of age, 18 males and females of each strain were given a daily dose of 215 mg/kg ETU, suspended in 0.5% gelatin, by gavage for 3 weeks. After this initial treatment, the mice were fed ETU at a concentration of 646 ppm in the diet until necropsied at 82-83 weeks of the experiment. Hepatomas were seen in 14 of the 16 male and in all the 18 female C57Bl/6 X C3H/Anf mice versus 8 of the 79 males and none of 87 females in the controls. In the C57Bl/6 X AKR mice, 18 of the 18 males and 9 of the 16 females developed liver tumors; the respective incidences in the controls were: 5/90 (male) and 1/82 (female). Slightly increased incidence of lymphoma (3/18, male; 4/16, female) was also observed in mice treated with ETU, compared to the controls (1/90, male; 4/82, female).

Other Thiourea Derivatives. Five thiourea derivatives, diethylthiourea (127), trimethylthiourea (128), phenylthiourea (129), dicyclohexylthiourea (130), and dithiobiurea (131) have recently been tested for possible carcinogenicity by the U.S. National Cancer Institute. The compounds were administered to groups of 50 Fischer 344 rats and $B6C3F_1$ mice at two dietary levels (approximately 1/2 maximum tolerated dose and maximum tolerated dose) for 77-109 weeks and observed for an additional period of 1-31 weeks. In the rat, diethylthiourea (125 or 250 ppm for 103 weeks) was carcinogenic, causing follicular-cell carcinomas of the thyroid in males (controls 0/18, low-dose 1/45, high dose 11/48) and follicular-cell adenomas or carcinomas of the thyroid in females (controls 0/18, low-dose 4/46, high dose 17/46). The increases in tumor incidence were statistically significant in the high-dose groups. Trimethylthiourea (administered as a mixture of 85% trimethylthiourea

and 15% dimethylthiourea at 250 or 500 ppm for 77 weeks) was also significantly carcinogenic (at the high dose) in females, inducing follicular-cell carcinomas of the thyroid (controls 0/17, low-dose 1/38, high-dose 14/47) but inactive in males. Phenylthiourea (60 or 120 ppm for 78 weeks) and dithiobiurea (6,000 or 12,000 ppm for 78 weeks) were not carcinogenic to rats at the doses administered; however, the mortality and weight gain data indicated that the high doses administered were not sufficiently close to the maximum tolerated dose. Dicyclohexylthiourea (25,000 or 50,000 ppm for 109 weeks) was also not carcinogenic in rats despite the fact that the compound induced an increased incidence of hyperplasia of the follicular cells of the thyroid. In the mouse, none of the five thiourea derivatives exhibited any significant carcinogenic effect. There was some suggestive evidence that dithiobiurea increases the incidence of hepatocellular carcinomas in female mice (controls 2/29, low-dose 8/47, high-dose 9/48); however, the evidence was not considered sufficient to establish the carcinogenicity of the compound. As in the rat, dicyclohexylthiourea increased the incidence of hyperplasia of follicular cells of the thyroid in the mouse.

5.2.2.8.3.3 THIOURACIL AND RELATED COMPOUNDS.

Thiouracil. Several investigators noted that thyroid tumorigenesis by TU in both rats and mice requires extremely long periods of treatment; the emergence of the tumors is preceded by diffuse hyperplasia and other preneoplastic changes. Following feeding 0.1% TU in the diet to 111 Stanford albino rats of both sexes for up to 45 weeks, Laqueur (138) observed diffuse and nodular thyroid hyperplasia (which was regarded as a benign lesion) in 33% of the animals. Similar observations were made by Money and Rawson (cited in ref. 112) and by Clausen (141) in Sprague-Dawley rats; adenomatous structures and

other types of changes described as neoplastic were found in the thyroid of the majority of the rats administered TU for 18 to 24 months. Unequivocal thyroid carcinoma, however, was seen only by Paschkis et al. (139) in 1 of 20 Sherman strain rats following treatment with 0.05 or 0.1% TU in the drinking water for 884 days. Eleven of the 20 rats so treated over 245 days were reported to have borne thyroid adenomas. The incidences of thyroid carcinomas and adenomas were enhanced to 15% and 94%, respectively, when the rats were also administered 0.03% 2-acetylaminofluorene in the diet (139). A study of Money et al. (140) in Sprague-Dawley rats showed that by the 500th day of treatment with 0.1% TU in the drinking water, all 81 animals developed thyroid adenomas.

As in many studies with rats, no histological evidence of neoplasia was found in the thyroid glands of mice ingesting TU at doses of 0.25-0.5% for a year or more, despite marked hyperplasia and extensive cellular alterations (120, 122, 188). However, Morris et al. (190) induced malignant autonomous thyroid carcinomas by means of serial transplantation of thyroid tissues to mice ingesting TU. The experiment was so designed as to provide conditions under which the thyroid tissue was exposed to TU beyond the lifetime of any one mouse. The study also illustrated the point that a long period of treatment is essential to the induction of thyroid neoplasms in mice by TU.

In studies on the effect of TU on adrenals of castrated C3H mice, Casas (142) found that almost 100% of the mice fed 0.3% TU in the diet for 10 months or more bore hepatomas. In subsequent studies with non-castrated C3H mice, a significantly high incidence of hepatomas was found in mice of both sexes administered a diet containing 0.3% TU for 18 months (12/13 in males and 14/16 in females) (143). When inbred TM strain mice were used, however, no hepatomas were seen in 44 TU-treated mice and 40 controls (143). The results led

the author to suggest that a genetic predisposition must exist for mice to be susceptible to hepatocarcinogenesis by TU. Hepatomas were also reported to occur in 6 of 21 castrated C57 X CBA mice fed 0.2% TU for 11-29 months (144).

Methylthiouracil. The production of thyroid neoplasms in rats, mice and hamsters following oral administration of methylthiouracil (MTU) has been repeatedly demonstrated by various investigators.

Hall (145) was the first to observe single adenomas of the thyroid in 3 of 12 Wistar rats receiving 0.01% MTU in the drinking water for 21-42 weeks. In a subsequent study, the observation period was extended to 94 weeks. Malignant tumors of the thyroid were found in 2 of 7 rats after 78 or more weeks of continuous treatment of MTU (146).

A series of studies were conducted by Doniach (147-149) using Lister rats. Methylthiouracil was given as a saturated solution in drinking water, prepared by suspending 1 g of the compound in each liter of tap water. In one experiment, solid and follicle adenomas of the thyroid were present in 10 of 16 rats sacrificed after 12-16 months (147). In two other studies, high incidences (19/20, 10/14) of thyroid adenomas were also found in rats killed after 15 months (148, 149).

Of 30 hybrid albino rats given 20 mg MTU in 0.2 ml. water by gavage (5 times a week for up to 100 weeks) 22 animals bore a total of 28 thyroid tumors (150). Nine of the tumors were malignant. In 24 Long-Evans rats fed 2.5 mg MTU in a low-iodine diet for 24-33 months, 8 had malignant tumors of the thyroid (154). Normal thyroid without any signs of hyperplasia was seen only in 2 rats. In 31 control rats given the low-iodine diet alone, none developed tumors; 25 of these thyroids were normal.

reported. Castration or thyroidectomy was shown to result in increased tumor incidence as well as shortened latent period of the tumorigenesis (159).

Only few studies have been conducted in hamsters on thyroid carcinogenesis by goitrogenic drugs. Thyroid adenomas were noted in hamsters given MTU at dose levels of 15 mg/100 g body weight (160) or 0.2% in drinking water (161) after 4-5 months of treatment. By the end of 12 months, 58% of MTU-treated hamsters developed thyroid adenomas of the papilliferous type; no such tumors were found in the controls (161). In animals simultaneously administered MTU and ^{131}I, greater tumor incidence and earlier appearance of adenomas and carcinomas were noted. On the basis of the high tumor incidence and short latent period for the development of thyroid neoplasia, it was suggested that hamsters might also be suitable models for research on thyroid tumor induction (161).

Propylthiouracil. In view of the tumorigenic effects of other goitrogenic compounds on the thyroid, the production of thyroid neoplasms in rodents by prolonged administration of propylthiouracil (PTU) is not unexpected. Indeed, PTU has been shown to induce thyroid adenomas and carcinomas in the rat, mouse, hamster and guinea pig.

When 0.2% PTU was administered in the diet to 48 female Wistar rats aged 2-15 months, single adenomas were observed in 24 animals following 2-14 months of treatment (163). The study indicates that the age of the rats is an important factor in the development of thyroid tumors induced by PTU. Older rats developed thyroid tumors with higher frequency and shorter latent periods than younger rats.

The induction of thyroid tumors in Wistar strain rats by PTU has been studied by Willis (162). Propylthiouracil was given in the drinking water for

up to 18 months. The initial dose (0.2%) was reduced to 0.1% at 3 months, to 0.05% at 6 months and to 0.025% at 12 months. In 48 rats surviving for 18 months, 31 developed adenomas and 7 had carcinomas of the thyroid. When PTU was given to rats in the drinking water at dose levels adjusted to give an intake equivalent to human doses (7 mg/kg body weight/day initially, then reduced to 1 mg/kg/day over a period of 3 months), high incidences of adenomas (50%) or carcinomas (17%) of the thyroid were observed in 18 rats which survived to the termination of the experiment at 18 months. Sellers et al. (164) have detected thyroid tumors in Wistar rats administered low doses of PTU (0.02%) alone in the diet or treated together with 0.02% sodium iodide or 0.02% dry thyroid powder for 15 months.

When 4 male A strain mice were fed 0.8% PTU in the diet for 18 months, all were found to have carcinomas of the thyroid and 3 had chromophobe adenomas of the pituitary gland (169). Multiple adenomas of the pituitary were also reported to occur in C57Bl mice maintained on a diet containing PTU levels of 1% or 1.2% for 17 months; the respective tumor incidences were 62% and 72% (170).

In 102 Syrian golden hamsters receiving 0.2% of PTU in the drinking water for about 14 months, 32 developed malignant lesions of the thyroid, among which 10 had metastasizing thyroid neoplasms (171).

Thyroid adenomas were also seen in 3 of 20 guinea pigs given 0.03% PTU in the drinking water for about 15 months. No tumors of the thyroid occurred in 10 untreated controls (172).

5.2.2.8.3.4 THIOACETAMIDE AND ETHIONAMIDE.

Thioacetamide. Fitzhugh and Nelson (115) studied the chronic toxicity of thioacetamide (TAA) in rats and was the first to suggest that TAA might be hepatocarcinogenic. In a 2-year toxicity test, groups of 10 albino rats were administered TAA at dietary levels of 0.1, 0.05, 0.025, 0.01, and 0.005%. At 0.01 and 0.005% dose levels, a hepatic cell adenoma in 1 of the 6 survivors and at 0.05%, a hepatocellular carcinoma was observed.

The development and morphology of the liver tumors in rats following prolonged feeding of TAA were later described in detail by Gupta (175, 176). One hundred fifty Wistar rats of both sexes were fed a diet containing 0.032% TAA. In 36 animals killed between 9-23 weeks, 18 were found to have bile duct tumors (175). Three hepatomas, one bile duct adenoma, and one hepatoma-cholangiocarcinoma with metastasis to the ovaries were found in 4 of 5 rats which ingested TAA for more than 47 weeks (176). Among 32 male rats which received similar treatment with TAA and survived for more than 16 weeks, 29 developed malignant tumors of the liver (173).

Recently, Dasgupta et al. (179) have detected 4 cases of hepatocellular carcinoma in 56 Wistar rats fed a diet containing 0.04% TAA. The tumors appeared after 300 days of TAA treatment and 2 of them metastasized to the lung.

Several other investigators (174, 177, 178) contributed to the evidence that TAA is a weak hepatocarcinogen toward rats. Moreover, Anghileri (178) and Shetty et al. (177) observed in the livers of rats biochemical alterations usually associated with hepatocarcinogenesis.

Swiss mice are particularly susceptible to the carcinogenic effect of TAA. Among 47 Swiss mice of both sexes maintained on a diet containing 0.03%

TAA, 6 of 6 males and 6 of 7 females killed 15 months after the beginning of treatment developed hepatocarcinomas. Some of the tumors were successfully transplanted in mice of the same strain (180). The progressive morphological, histological, and biochemical changes in the liver of mice during TAA-induced hepatocarcinogenesis have been described in some detail (180, 192).

Hamsters appear to be refractory to the carcinogenic effects of TAA. No liver tumors were found in 10 male and 10 female Syrian golden hamsters given 25 mg TAA by gavage once weekly for 30 weeks (181). The few tumors occuring in various organs were not considered by the authors to be related to treatment with TAA.

Ethionamide. The tuberculostatic drug, ethionamide, was among the chemicals selected for carcinogenicity bioassay by the U.S. National Cancer Institute (182). The compound was fed to 35 Fischer 344 rats and 35 B6C3F1 mice of each sex 5 days/week for 78 weeks at the following doses: 1,500 or 3,000 ppm to rats and 1,000 or 2,000 ppm to mice. The incidences of tumors in various organs were found not to be significantly different from those of the untreated controls. However, in a recent review, Biancifiori (183) stated that papillary and epidermoid carcinomas of the thyroid are obtained in mice by the administration of ethionamide.

5.2.2.8.3.5 MODIFICATION OF CARCINOGENESIS.

A number of exogenous chemical agents are capable of modifying the carcinogenicity of thiocarbonyl compounds. Conversely, several thiocarbonyl compounds have been shown to suppress the spontaneous incidence of mammary tumors in rodents and inhibit the carcinogenicity of other chemicals. Examples of such interactions are outlined below.

Bielschowsky (13, 14) reported that combined treatment of 2-acetylaminofluorene and allylthiourea induces a significant number of thyroid tumors in rats. Allylthiourea alone is also carcinogenic, but its effect is much less pronounced than that of the combined treatment. 2-Acetylaminofluorene alone has no discernible effect on the thyroid. The results suggest that 2-acetylaminofluorene may potentiate the carcinogenic effect of allylthiourea toward the thyroid.

As may be expected from the goitrogenic activity of low-iodine diet, the carcinogenicity of thiocarbonyl compounds toward the thyroid may be potentiated by low-iodine diets or by the administration of radioactive iodide. Israel and Ellis (158) showed that C57 mice fed a stock diet and given 0.05% MTU in the drinking water for 480 days do not develop thyroid tumors despite marked hyperplasia. However, of the 25 MTU-treated mice that were kept on a low-iodine diet, 11 were found to bear papillary adenomas and one had an adenocarcinoma of the thyroid. Jemec (156) reported that C3H mice given 0.2-0.5% MTU in an iodine-poor diet developed a significantly higher incidence of thyroid tumors (30.7%) than those given 0.1% MTU in drinking water and kept on an iodine-rich diet (1.2%). The thyroid tumor incidence in the iodine-poor and iodine-rich controls were 0.7% and 0%, respectively. The results are suggestive of potentiation of MTU by low-iodine diet; however, it is not known to what extent the potentiation may have been due to the different dose levels of MTU used in the iodine-poor and iodine-rich diets.

It is interesting to note that although high-iodine diet may inhibit the carcinogenic effect of thiocarbonyl compounds toward the thyroid, potassium iodide may potentiate the carcinogenicity of PTU if the two agents are given alternatively to produce hyperplasia and involution in repeated cycles. Zimmerman et al. (166) showed that in a group of 15 albino rats given 0.1% PTU

in the drinking water for one year, four displayed single adenomas of the thyroid. However, when rats were maintained alternatively on PTU to produce hyperplasia, and on potassium iodide (0.01% to involute the thyroid), a much higher incidence (17/29) of thyroid tumors was encountered. It was hypothesized that nodular goiter is the result of repeated cycles of thyroid hyperplasia and involution; apparently, the same treatment also favors tumorigenesis.

The investigation of Willis (162) indicates that ^{131}I in conjunction with PTU increases the incidence of thyroid carcinomas and shortens the latent period. The combined effect of PTU and radioactive iodine was also investigated by Lindsay et al. (167) using weanling male Long-Evans rats. Both PTU and ^{131}I appear to play a part in the initiation and promotion of thyroid carcinogenesis and their effects are additive. Groups of rats were given a single intraperitoneal injection of ^{131}I and/or 0.1% PTU in the diet for a year. Thyroid adenomas occurred in about 10% of the rats injected with ^{131}I and in 48% of the rats fed the PTU-containing diet. The incidence of thyroid tumors in the rats treated with the combination of ^{131}I and PTU was, on the other hand, 65%. Al-Hindawi et al. (168) did not observe thyroid neoplasms in rats which received ^{131}I at the level which was 1/100th that used by Lindsay et al. (167) for 7-9 months. However, Al-Hindawi et al. (168) did observe a 100% incidence of thyroid tumors in rats treated with the radioactive iodine and PTU (60 ug/ml in drinking water) simultaneously. High incidence (5/18) of invasive carcinomas of the thyroid with metastasis to the lung were reported in Wistar rats receiving both ^{131}I and PTU and observed for 6-15 months (165).

The suppression of spontaneous mammary tumors by thiourea is illustrated by the investigations of Morris et al. (193) who reported that only 17% of 42

virgin female C3H mice treated with 0.375-0.5% thiourea in the diet had spontaneous mammary tumors, as compared to 94% of 52 untreated animals after 18 months. Similarly, Vazquez-Lopez (194) found that 6-month administration of thiourea in drinking water reduced the incidence of spontaneous mammary tumors in virgin and breeding C3H mice from 40/96 to 5/85. Like thiourea, TU also inhibits the development of spontaneous mammary tumors in mice; Morris et al. (195) reported that while 48% of 62 control strain C mice developed mammary tumors, no neoplasms were found in 62 mice fed 0.375 and 0.5% TU in the diet until death. Treatment with TU also reduced the incidence of spontaneous mammary tumors in C3H mice from 92% to 19%. Moreover, a lengthening of the latent period was noted in mice following TU treatment (196). Suppression of chemically induced mammary carcinogenesis by thiocarbonyls has also been noted; chronic administration of PTU inhibits the development of mammary tumors in rats induced by 7, 12-dimethylbenz[a]anthracene (197-199) or 3-methylcholanthrene (200). It was suggested that the suppression of mammary tumorigenesis by thiocarbonyls is brought about by reduction of the calorie intake, which has been shown to affect the growth of the mammary gland (199). An alternative explanation involves the hormonal imbalance resulting from the hypersecretion of thyrotropic hormone (TSH) in response to the low thyroxine level caused by thiouracils. Because of the abnormally large production of TSH, the secretion of hormones related to the development of mammary tumors would be decreased (193, 196, 198).

The possible synergistic and antagonistic effects of thiocarbonyl compounds with other chemicals in hepatocarcinogenesis have been studied by various investigators. Deichmann and coworkers (201, 202) fed Osborne-Mendel rats diets containing 50 or 80 ppm each of thiourea, aramite, methoxychlor and DDT for 2 years. The incidences of tumors in the rats given the four com-

pounds in the diet were not consistently higher than those in the rats given the compounds singly. Similar negative results were obtained when groups of rats were fed the compounds singly, each at a dose representing 50% of their liver tumor-inducing dose (thiourea, 50 ppm; aramite, 200 ppm; methoxychlor, 1,000 ppm; DDT, 200 ppm), and in combination. These findings led the authors (202) to conclude that the four compounds do not exert a synergistic or additive tumorigenic effect in the rat.

The combined effect of TU and 2-acetylaminofluorene (2-AAF) on hepatic tumorigenesis in rats was studied by Paschkis et al. (139, 203) and by Leathem and Barken (204). Eighty-four percent of Sherman rats receiving 2-AAF (0.03% in the diet) alone developed hepatomas, while the incidence of hepatomas was 22% in animals receiving 2-AAF and TU (0.05 or 0.1%, respectively, in drinking water) in combination (139). In subsequent studies this observation was confirmed. While hepatomas were induced in 9 out of 9 rats by 2-AAF, only 1 of 16 animals administered 2-AAF + TU developed such tumors (203). Similarly, the incidence of hepatomas decreased from 50% in rats fed 2-AAF alone to 12.5% in rats which ingested 2-AAF + TU (204).

Simultaneous administration of TU also protects the rats against liver tumorigenesis by 4-dimethylaminoazobenzene. Liver tumors were found in 46% of female Sherman rats given both 4-dimethylaminoazobenzene and TU, but in 88% of rats administered 4-dimethylaminoazobenzene alone (139). It has also been reported that TU treatment significnatly inhibits the development of cholangiocarcinomas induced by diethylnitrosamine in gerbils (205). Besides TU, thiourea has been shown to inhibit carcinogenesis by other chemicals. Gorbman (120) reported that while 30% of strains A and C57 mice developed local carcinomas and squamous cell carcinomas between 90 and 130 days after a single subcutaneous injection of 1 mg benzo[a]pyrene, no tumors were found in

mice receiving a mixture of 2% thiourea and 1 mg benzo[a]pyrene in the diet after 200 days of age. Thiourea is also believed to suppress the carcinogenic action of concomitantly administered 2-AAF in the mouse (120).

The mechanism of protection by thiocarbonyl compounds against the carcinogenic action of 2-AAF and other carcinogens is unknown. Paschkis et al. (203) have shown that the protective effect of TU is not related to the induced hypothyroidism. The suggestion has also been made that thiouracils may act upon enzymes in the liver which convert carcinogenic chemicals to inactive forms (139). They may also act as antimetabolites, interferring with the uptake of uracil, possibly a nutritional requirement for the emergent cancer cells induced by 2-AAF (203).

5.2.2.8.4 Metabolism and Mechanism of Action.

5.2.2.8.4.1 TISSUE DISTRIBUTION AND METABOLISM.

Thiourea. Studies conducted in rats and man have shown that thiourea is rapidly absorbed from the gastrointestinal tract and distributed in whole body tissues (206). The distribution is not uniform but displays marked variations in the tissues and body fluids. According to Schulman and Keating (207), when ^{35}S-thiourea is injected intraperitoneally into rats, notably higher radioactivity is found in the thyroid and kidney than in other tissues. In contrast, Maloof and Soodak (208), reported that the thyroid takes up only a small fraction of the administered ^{35}S-thiourea, but is very effective in the metabolism of the compound. The radioactivity in the thyroid is primarily in the form of ^{35}S-sulfate. Marked decrease in the metabolism of ^{35}S-thiourea was noted in the thyroid of hypophysectomized rats, indicating that pituitary hormones are important in regulating the metabolism of thiourea.

Thiourea is excreted rapidly by the kidney (206, 207). Within 48 hours following its administration, 98% of the radioactivity appeared in the urine mainly as undegraded ^{35}S-thiourea and, to a much lesser extent, as ^{35}S-sulfate and ^{35}S-ethereal sulfate (207). Negligible amounts of radioactivity was excreted in expired air or in the feces (206, 207).

Phenylthiourea. Following oral administration of ^{35}S- or ^{14}C-labeled phenylthiourea to rabbits, 80-86% and 8-10% of the radioactivity were found in the urine and in the feces, respectively, within 48 hours. The urinary metabolites were identified as: sulfate (62% of ^{35}S-phenylthiourea), phenylcarbamic acid glucuronide (30% of the ^{14}C-phenylthiourea), p-hydroxyphenylthiourea (16%), p-hydroxyphenyl urea (14%), phenylthiourea (6%), phenylurea (4%), phenylcyanamide (1%), aniline (4%), and urea (3%). The metabolism of phenylthiourea was similar in the rat (25).

Ethylenethiourea (ETU). The distribution, excretion, and metabolism of ETU have been investigated in the rat (104, 209-214), mouse (104, 215), guinea pig (213) cat (214), and cow (216). Like thiourea, ETU is readily absorbed from the gastrointestinal tract and distributed rapidly in various tissues, and in the fetus (209, 210). The compound is distributed uniformly in most tissues except the thyroid, which showed significantly higher concentrations of ETU (209-211, 213). The $t_{1/2}$ of ETU elimination was about fivefold higher in the thyroid than in other tissues (211).

Ethylenethiourea was eliminated rapidly through the urine. In rats, 72.8% of the total radioactivity was recovered in the urine within 24 hours after the administration of ^{14}C-ETU; at least 95% of the excreted radioactivity was unchanged ETU (209, 211). The metabolites of ETU have been identified as ethyleneurea (210, 212, 214), imidazolone (212, 214), imidazoline

(214), thioimidazole, thiohydantoin, N-methyl-ETU and N-methyl-thioimidazole (212). Kato et al. (210) reported that ETU is also metabolized to CO_2 via desulfuration followed by oxidative opening of the imidazolidine ring between C_4 and C_5, which is followed by decarboxylation of the 1,3-dicarboxyurea formed.

While undegraded ETU is the predominant product in the urine of rats, only 40-50% radioactivity in the urine of mice represents unchanged ^{14}C-ETU, indicating that ETU is metabolized to a greater extent in the mouse than in the rat (104, 215). The desulfurated derivative, ethyleneurea, accounts for approximately 12% of the radioactivity excreted in the urine of mice (215). The only other identified metabolite of ETU in mice is 2-imidazolin-2-yl sulfenate (217).

Of 80% of the radioactive materials excreted in the urine of cats 24 hours after the administration of ^{14}C-ETU, 64% is S-methyl-ETU, 4% is ethyleneurea, and only 28% represents the undegradated parent compound (214). In cows, ETU is metabolized to ethyleneurea, ethylenediamine, oxalic acid, glycine, and urea (216).

In vitro studies on ETU, thiourea and phenylthiourea show that the compounds are metabolized by the microsomal FAD-containing monooxygenase from pig liver to sulfinic acid and formamidine sulfinic acid (218).

Thiouracil and Derivatives. These compounds are rapidly absorbed from the gastrointestinal tract in the rat and in man (219, 220) and can be found in essentially all tissues and body fluids (219, 220). In vivo studies in rats with ^{35}S-labeled thiouracils showed significantly higher accumulation of ^{35}S-radioactivity in the thyroid than in the plasma (220-223). Compared to thioureas, thiouracils are less rapidly metabolized in the thyroid and only a

small fraction is converted to ^{35}S-sulfate (208, 221). Among the thiouracils, unmetabolized ^{35}S-PTU accumulates in the thyroid the most and ^{35}S-TU the least, following administration of equimolar doses (220, 222). In addition to the intact drugs and ^{35}S-sulfate, other radioactive materials detected in the thyroid were protein-bound ^{35}S-compounds and unknown metabolites (221, 223).

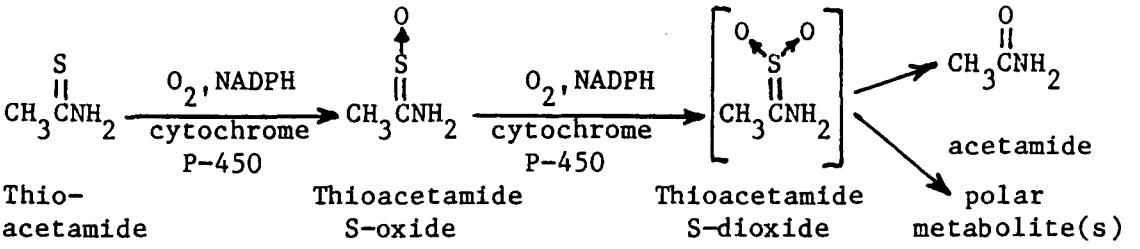
The rate of metabolism in other tissues is faster with TU than with PTU (220). In rats, about 80% of the total radioactivity appeared in the urine within 24 hours after the administration of ^{14}C-TU (224). In vitro studies suggest that the metabolism of TU involves initial desulfuration with the formation of uracil which is then cleaved to produce β-alanine, ammonia, and CO_2 (224). Alternatively, TU may be converted to thiouridinemonophosphate and thiouridinetriphosphate by enzyme systems of liver and thyroid similar to those involved in the synthesis of UTP from uracil (225-227).

When ^{14}C-PTU was administered to rats either by gavage or parenterally, 75-90% of the administered radioactivity was excreted in the urine and about 15% in the bile during the first 24 hours (228). Analysis of the 24-hour urine sample showed that propylthioglucuronide accounts for 40-48% and intact ^{14}C-PTU for 9-15% of the administered dose (228, 229). Three other urinary metabolites: sulfate, propyluracil, and S-methyl-PTU were identified (229). Similar metabolic products were found in the bile (229).

In guinea pig, a urinary metabolite believed to be PTU-disulfide was identified. Other metabolites in the urine and the bile remain unidentified (230).

Thioacetamide (TAA). Thioacetamide is rapidly metabolized in rats. Following subcutaneous injection of ^{35}S-labeled TAA, more than 80% of the radioactivity is excreted in the urine within 24 hours, of which approximately

25% was unchanged TAA (231). The major metabolic products are inorganic sulfate (231, 232), TAA-S-oxide (232, 233), and acetamide (234). In vitro studies indicate that TAA is metabolized by the hepatic mixed-function oxidases (235, 236) to TAA-S-oxide, which is then further metabolized by the microsomal monooxygenases or amine oxidase to acetamide and other polar products (236, 237). The metabolic pathways of TAA in the rat liver are as follows (236):



Ethionamide. The ready biochemical interconversion between ethionamide and its sulfoxide has been established in mice, rats, dogs (238), and humans (238-240). The two compounds are rapidly absorbed from the gastrointestinal tract and considerable amounts of both compounds appear in the blood within 15 minutes, regardless of which compound is administered (238). Only small amounts of unchanged ethionamide and its sulfoxide are found in the urine of animals or humans receiving ethionamide. Several urinary metabolites have been detected. These are 2-ethylisonicotinamide, 2-ethylisonicotinic acid, and inorganic sulfate (238, 241), as well as metabolic products suspected to be pyridones (238, 242).

5.2.2.8.4.2 MECHANISMS OF ACTION.

Thiourea, Thiouracil, and Derivatives. The mechanism of carcinogenesis by thiourea, TU and their derivatives are not clearly understood. It is

generally believed that the modes of carcinogenic action of these substances are identical, at least in thyroid tumorigenesis: they act indirectly by causing hormonal imbalance resulting from an altered thyroid-pituitary relationship. As known from their goitrogenic effects, they act by inhibiting the synthesis of thyroxine; the resulting decrease in the level of thyroxine then evokes an increased release of thyrotropic hormone (TSH) from the pituitary to act on the thyroid epithelium, which subsequently becomes hyperplastic and neoplastic. Several studies have demonstrated that a high level of pituitary thyrotropic hormone is essential for the formation and growth of thyroid tumors (126, 167, 168, 189). Thyroid tumors are not found in hypophysectomized animals subjected to carcinogenic goitrogens (243).

The inhibitory effect of thiourea and related compounds on thyroxine synthesis may be due to direct inhibition of the peroxidation system responsible for the conversion of iodide to iodine, thus inhibiting the iodination of tyrosine in the production of thyroxine (244, 245). It was also suggested that these compounds may bind iodine and reduce the level of free iodine available for reaction with tyrosine (220, 246). However, since 6-amino-TU, which also reacts with iodine, exhibits little antithyroid activity (247), the biological activities of thiourea, TU and related compounds appear to be due to their inhibitory effects on thyroid peroxidase, rather than to the binding of iodine. Propylthiouracil also inhibits the monodeiodination of thyroxine (T_4) to triiodothyronine (T_3) (248-250).

The metabolism of thiourea by the thyroid of rats is decreased after hypophysectomy. Yet, thiourea is as effective in inhibiting the formation of protein bound iodine in the thyroid of hypophysectomized rats as in the thyroid of non-operated animals, suggesting that metabolism is not essential for the effect (208). Similarly, the antiperoxidase and antithyroid activ-

ities of all known metabolites of TU (227) and PTU (229) are much lower than those of TU and PTU themselves indicating that metabolism is unlikely to be involved in the mechanism of action of these thiouracils.

The relatively high accumulation in and slow disappearance from the thyroid of unmetabolized TU compounds may explain their selective action on the thyroid. A correlation between the level of unmetabolized TU, MTU, and PTU accumulated in the thyroid of rats and their goitrogenic potency has actually been demonstrated (222). However, in studies of patients treated for thyrotoxicosis, the concentration of thiouracils in the thyroid was found not to be associated with the potency of these drugs (220).

The induction of cancer in organs other than the thyroid has led several workers to suggest that thiourea compounds may also act as direct-acting carcinogens, although the mechanism is totally unknown (116, 118, 124). The positive results observed in several mutagenicity assay systems with thiourea and related compounds (see Section 5.2.2.8.2.2) appear to support this view. The S-oxygenation of several thioureas to sulfinic acids by microsomal FAD-containing monooxygenase has been suggested to be an important pathway in their activation to toxic, reactive metabolites (218). Hollinger and coworkers (251, 252) have reported the binding of ^{14}C-thiourea to proteins in rat tissues; the binding was, however, more extensive in the lung (which is not a target of the carcinogenic action of thiourea) than in other tissues. A toxic effect which may be accounted for by this binding is the production of edema in the lung by thiourea (251).

If hormonal-imbalance is the cause of thyroid cancer induced by thiourea and its derivatives, conceivably information on the goitrogenic activity of these compounds is important for the assessment of their carcinogenicity. The

relationship between goitrogenic activity and the structure of these chemicals has been discussed (see Section 5.2.2.8.2).

Thioacetamide. Although the liver is the major target organ of thioacetamide (TAA) toxicity and carcinogenicity, it does not accumulate higher levels of the compound than other tissues (231). This observation has led to the suggestion that the striking specific action of TAA may be due to the hepatic metabolism of TAA to reactive intermediate(s) (34, 35, 231). Comparison of the toxicity of TAA and of one of its known metabolites, TAA-S-oxide, showed that the latter produced a more severe hepatic necrosis than equivalent doses of TAA (235). Thioacetamide-S-oxide has also been stated to exhibit marked carcinogenic effects (cited in ref. 253). However, TAA-S-oxide is not considered to be the ultimate carcinogenic metabolite of TAA and it is believed to undergo further metabolism to reactive metabolite(s) (232, 235, 236). Recently, in vitro binding of TAA-S-oxide to calf thymus DNA in the presence of rat liver microsomes has been shown (237). The binding reaction requires NADPH, and is inhibited by CO and by an antibody of rat liver cytochrome P-450, indicating that a microsomal cytochrome P-450 requiring mixed-function oxidase is involved in the metabolic conversion of TAA-S-oxide to reactive intermediate(s) which then bind to calf thymus DNA. The chemical nature of the reactive intermediate(s) is not known. The investigations of Porter et al. (232) indicate that the methyl group and the thiocarbonyl carbon atom, but not the sulfur atom, of TAA or TAA-S-oxide is directly involved in the binding with tissue macromolecules (232).

Various cytological and biochemical alterations have been reported to be associated with TAA hepatocarcinogenesis (35, 177, 254-256). Modification of the nuclear envelope of liver cells following TAA administration is receiving increasing attention. The swelling of the nuclei and the increase in phos-

phatidylserine in the nuclear envelope have been hypothesized to affect cellular homeostasis and alter chromatin structure (254, 255).

5.2.2.8.5 Environmental Significance.

Thiocarbonyl compounds have a wide variety of applications; human exposure may occur under various circumstances. Some of these compounds are of clinical value for the treatment of thyrotoxicosis and in other therapeutic applications. Others find use in the industry and agriculture while some are of research or toxicological interest. The production and use of thiourea, ETU, TU, MTU, and PTU have been extensively reviewed by an International Agency for Research on Cancer Working Group (155).

Thiourea. Thiourea is commercially available and several synthetic methods are used in its production. The chemical also occurs naturally in the seeds of shrubs belonging to the genus Laburnum (257) and as a metabolite of Verticillium albo-atrum and Bortrylio cinerea (155). Thiourea was or has been used as an antithyroid agent, fungicide, intermediate in the production of fireretardant resins for fabrics, as a dye-bath adjuvant of textiles, as vulcanization accelerator, and as an anti-yellowing agent in the production of diazo-type coatings for copy paper. The chemical is also useful in the photographic industry, in cosmetic hair preparations, in the dry-cleaning industry, and in the synthesis of pharmaceuticals, insecticides and other organic compounds (cited in ref. 155). Although many of its applications in the industry are still continuing, the use of thiourea as an antithyroid drug, fungicide, and food additive has long been discontinued in many countries including the United States, because of its potential hazard to humans (155). The chemical decomposes slowly to sulfate and ammonia under the action of microorganisms in soil and sewage sludge (cited in ref. 258).

Ethylenethiourea. Ethylenethiourea is a major degradation product of the metal salts of ethylenebisdithiocarbamic acid, which are extensively used as fungicides in the management of diseases of agricultural crops. Amounts up to 14.5% of ethylenethiourea (ETU) have been reported in 28 different commercial formulations containing maneb, zineb, or mancozeb after 39 days of storage under controlled conditions of elevated temperature and humidity (259, 260). Trace levels of ETU residues have also been detected in various vegetables, fruits, and crops field-sprayed with these formulations (261-265). There is general agreement that ETU decomposes rapidly in plants, soils and water, and that accumulation of significant amounts of ETU on the crops or in the environment is unlikely (266-269). However, home cooking or commecial processing of certain foods that contain residues of the ethylenebisdithiocarbamic acid metal salt fungicides have been reported to cause increases in the level of ETU in foods (263, 270, 271).

Human exposure to ETU can also be brought about by its applications as accelerator in the vulcanization of various elastomers, and as intermediate in the manufacture of dyes, synthetic resins, antioxidants, and pharmaceuticals (272). The U.S. National Institute for Occupational Safety and Health estimated that approximately 17,000 employees in 24 different occupations were potentially exposed to ETU in the workplace between 1972 and 1974. The major routes of occupational exposure are inhalation and skin contact (272).

Other Thiocarbonyls Compounds. Both diethyl- and trimethylthiourea are used as vulcanization accelerators in the production of various types of rubbers. Diethylthiourea is also used as an inhibitor of corrosion in metal pickling solutions. Other applications of trimethylthiourea include uses such as inhibitor of ozone fading of polyamide dyes, as a component of adhesives, and as an intermediate in organic synthesis. Dithiobiurea is used as a fuel

in pyrotechnic disseminating compositions and electroplating baths for metals. It is also an important compound for the photographic industry. Phenylthiourea is used occasionally as a rodenticide. The chemical is also employed as a test agent in medical genetics (273), since the ability to taste phenylthiourea is an inherited trait. These thiocarbonyl compounds were produced and marketed in quantities in excess of 1,000 pounds annually in the United States (cited in refs. 127-129, 131). Dicyclohexylthiourea is primarily used as a laboratory reagent in biochemical and physiological research (130).

Thiouracil, MTU, and PTU have been used as antithyroid drugs in human and veterinary medicine (155). Thiouracil is also effective in the treatment of angina pectoris and congestive heart failure. In animal husbandry, MTU and PTU were used for promoting the growth and fattening of animals. However, these applications of thiouracils appear to have been discontinued in the United States (155).

Thioacetamide is widely used as a substitute for hydrogen sulfide in the analytical chemistry of heavy metals (273). Ethionamide is among the drugs of choice in the treatment of tuberculosis. The drug is administered to adults at dose level as high as 1 g daily (19).

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Notes Added After Completion of Section 5.2.2.8

The presence of an intact sulfur atom [not bearing oxygen atom(s)] in the molecule of ethylenethiourea is essential not only for its teratogenic action (see Section 5.2.2.8.2), but also for its mutagenicity. This conclusion is drawn on the basis of recent observations that the metabolite formed by oxidation of the sulfur in ethylenethiourea does not exhibit any mutagenic activity in Salmonella typhimurium TA1950 or in the host-mediated assay in mice (1). In contrast, thioacetamide-S-oxide, but not thioacetamide itself, shows mutagenicity in the Ames test without the S9 mix (2).

Hepatocarcinogenesis induced by thioacetamide has been studies in inbred male ACI rats which do not have a spontaneous liver tumor incidence (3). Administration of 0.035% thioacetamide in a semipurified diet to the rats for 1 year resulted in the development of primary hepatocarcinomas and cholangiocarcinomas. The tumor incidence and type were strongly influenced by the dietary conditions.

Thiobenzamide (C_6H_5-$CSNH_2$), a thiono compound structurally related to carcinogenic chemicals of this group, was reported to induce hyperplastic nodules and tumors in the liver of rats (4). Additional studies (5) suggest that thiobenzamide acts principally as a promotor in liver carcinogenesis.

References for Section 5.2.2.8 Update

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