IV. BIOLOGIC EFFECTS OF ETU

A. Toxicity

Acute and Subacute Effects

No description of the acute effects of large doses of ETU in humans was found in the literature. Ingestion of any significant amount of ETU is unlikely because of its bitter taste.

Stanley and Astwood (1947) investigated ETU as one of a group of thiol compounds being considered as possible therapeutic agents to suppress the action of the human thyroid gland. A single administration of 100 mg/kg of body weight (BW) to one volunteer gave a response of 2 when inhibition of radioactive iodine uptake was graded on a scale of 0 to 5. The authors estimated the activity of ETU to be 50% of that of thiouracil, an antithyroidal agent used clinically at that time (1940-49). Details of the study were not reported with respect to ETU.

McGinty and Wilson (1949) investigated the depression of radioactive iodine uptake by the thyroid gland when thiol compounds were administered to rhesus monkeys. One compound tested was ETU. Four monkeys were dosed at 5, 10, 10 and 40 mg/kg BW. At doses of 10,10 or 40 mg/kg BW, radioactive iodine uptake by the thyroid was inhibited for at least 24 hours. The authors referred to the response at 5 mg/kg BW as "suggestive". However, inspection of a graphic display of the data did not reveal any difference in response between the 5 or 10 mg/kg BW doses. No further details were reported. The authors estimated ETU to be two times as active as thiouracil. Since it has been shown that the

uptake of radioactive iodine by the thyroid gland is, in general, related to the antithyroidal action of a compound (Stanley & Astwood, 1947), ETU probably exerts an antithyroidal action in man. No reports of clinical testing of ETU have been found in the literature, and neither of the above authors recommended ETU for clinical testing as a thyroid-suppressing drug.

Numerous studies in experimental animals have been done on the acute and subacute effects of ETU (Davis et al, 1973; Graham et al, 1970; Meyer et al, 1949; Seifter et al, 1948; Freudenthal et al, 1977).

The acute and subacute effects of ETU are similair to other substituted thioureas. The primary effect is on the thyroid gland at doses which do not cause death. However, the acute cause of death in rats is lung edema. (Dieke et al, 1947; Khera and Tryphonas, 1977). The LD-50 for ETU is reported as 1832 mg/kg BW in the rat (Graham et al, 1972).

The subacute effects of ETU on the thyroid of rats were studied by Graham et al (1973). This study was well planned and carefully executed. In six groups of male Charles River Sprague - Dawley rats fed ETU for 2, 6, and 12 months at levels of 0, 5, 75, 125, 250, and 500 ppm in the diet, 5 micro curies of 131-I were administered to determine iodine uptake. Iodine uptake was determined at 24 hours. Although the uptake was variable in the controls, 131-I uptake, as measured by counts per minute per mg of thyroid tissue, was significantly decreased at the 25, 125, 250, and 500 ppm level of ETU for all of the feeding periods. Other factors, such as body weight gain, histologic appearance of the thyroid gland, and thyroid gland to

body weight ratio, all corroborated the fact that when ETU is fed to rats in the diet at levels above approximately 100 ppm, there is a dose-response decrease in thyroid function (see Tables 1-3, Appendix D).

Seifter et al (1947) investigated the subacute effects of ETU in rats when administered at 0.1% of the diet. They found normal weight gain was depressed, the organ-to-body-weight ratio of the thyroid increased, and the histologic appearance of the thyroid was hyperplastic. They also concluded that the thymus size and appearance was a very sensitive indicator of the extent of subacute exposure to ETU, since the weight of the thymus gland was much reduced, and the histological appearance of the gland was altered when ETU was administered.

Freudenthal et al (1977) estimated the "subacute no effect level" of ETU (when given for 90 days to rats) to be 25 ppm in the diet. At this level, he was not able to detect a biologic response to ETU, and suggested this to be a "no effect" level (see Tables 4-6, Appendix D). A twofold increase occurred in Thyroid Stimulating Hormone (TSH) at 30 days at 625 ppm in the diet; thyroxine (T-4) levels reflected these changes in TSH, but triiodothyronine (T-3) did not. T-3 in females was significantly increased at 125 ppm in the diet for 30 days when a decrease was expected. These results indicate that ETU may have a variable effect on thyroid function during the first 3 months of intake, but, in general, inhibition is the main effect (see Tables 3-5 in Appendix D).

Chronic Effects

While no descriptions of ETU toxicity in humans are available in the literature, the chronic effects of ETU poisoning that may be expected in humans, on the basis of the actions of similar antithyroidals, are given in complete clinical descriptions by Astwood (1970) and Labhart (1976). Basically, these signs (comprising a syndrome termed myxedema) include drying and thickening of the skin, an unusual puffy swelling of subcutaneous tissues (termed non-pitting edema), a yellowish or ivory pallor to the complexion, an enlarged tongue, a husky voice, and dry brittle hair. Since, in myxedema, all metabolic rates are decreased, mental processes, talking, pulse rate, and breathing may also be slowed (Labhart, 1976).

The chronic effects of ETU in experimental animals are especially related to effects on the thyroid. Graham et al (1975) reported that when ETU was fed to rats in the diet at a rate of 250 to 500 ppm, the organ-to-body-weight ratio for the thyroid gland increased significantly (see Table 2 in Appendix D). While this overt manifestation of increased size of the thyroid occurred only at levels of 250 ppm or 500 ppm ETU in the diet, histomorphological changes in the thyroid occurred at all dose levels tested. The lowest dose of ETU tested in rats by Graham was 5 ppm in the diet. Hyperplasia of the thyroid occurred at this lowest dose.

ETU, propylthiourea, methimazole, and other thiourea based antithyroidals do not decrease the iodine trapping ability of the thyroid but rather block the formation of the hormones thyroxine (T-4) and triiodothyronine (T-3). They do so by inhibiting the action of

thyroid peroxidase. When ETU and other antithyroidals are administered to man or experimental animals, the preformed T-4 and T-3 continue to be secreted. As the supply of T-4 and T-3 is exhausted in the thyroid gland, blood concentration decreases. This results in increased secretion of thyroid stimulating hormone (TSH) from the pituitary gland which produces a hyperplastic, highly vascularized thyroid gland that can then trap iodine more efficiently. However, this compensatory mechanism is insufficient, since the formation of T-4 and T-3 is still held in check by the "poisoned" thyroid oxidase enzyme, and eventually the individual becomes myxedematous (Labhart, 1976).

An increased level of cholesterol in the blood is common in myxedematous patients (Labhart, 1976). Gak (1976) reported that ETU produced hypercholesterolemia in both hamsters and rats at 5 ppm dietary levels. The lowest level at which ETU may produce elevated cholesterol in the blood is unknown, since 5 ppm of ETU in the food was the lowest dose administered in this investigation.

Bone marrow suppression has been noted in humans by Martelo et al (1967) following treatment with the structurally similar antithyroidal substance propyl thiouracil, and should be considered as a possible consequence of chronic human exposure to ETU. However, original data retained by Dr. Graham during investigation of the carcinogenicity of ETU (Graham, 1973) and supplied to NIOSH in 1977, did not reveal any significant consistant changes in the total or differential white blood cell counts in rats exposed to ETU. Bone marrow suppression was not noted when antithyroidal substances were first tested in experimental animals and was discovered to be a consequence of antithyroidal therapy

only after extensive clinical usage (Labhart, 1976). The relative incidences of agranulocytosis following the use of various antithyroidals may be found in "AMA Drug Evaluations" (1973). Other adverse reactions found in this class of antithyroidals include hypersensitivity reactions, such as rash, arthralgia, loss of taste, etc. A more complete discussion of adverse reactions to antithyroidals can be found in "AMA Drug Evaluations" (1973).

B. Biokinetics

Lyman (1971) administered ETU to cows and demonstrated ETU and the following metabolites in the urine: ethylene urea, ethylene diamine, oxalic acid, glycine, and urea. Unchanged ETU was also found in the milk.

In a study designed to investigate the teratogenic effects of ETU, Ruddick et al (1976) reported that when ETU was fed to pregnant rats, 72.8% of the dose (240 mg/kg BW) was excreted in the urine within 24 hours. The blood level peaked at 2 hours and rapidly fell to unmeasurable concentrations by 96 hours. Less than 2% of the ETU was metabolized to other substances which included ethylene urea. It was concluded by the authors that ETU is degraded to only a small extent in the rat.

From the information gained regarding the biokinetics of ETU, it may be expected that kidney failure or substances interfering with urinary excretion may prolong the effects of ETU taken into the body. The results of these metabolic studies also indicate that carcinogenic

effects most likely result from the chemical ETU itself rather than from a metabolic product, since ETU is not metabolized extensively and none of the known metabolic products are suspect carcinogens nor structurally related to known carcinogens (Ruddick, 1976).

C. Carcinogenic Effects

Five independent groups of investigators have reported ETU to be carcinogenic in animals.

In 1969, Innes et al, using mice, reported hepatoma from ETU administration (lifetime dose of 646 ppm in the diet) in 32 out of 34 males versus 13 out of 169 in controls. Control females had 1 hepatoma in 169 mice, while those given ETU had 27 hepatomas in 34 necropsied (see Table 6, Appendix C).

Compared to seven grouped positive control carcinogens, including ethylene imine and ethyl carbamate (urethane), ETU was on the average 6.85 times more potent in producing hepatomas in the males, and 3.17 times more potent in the females. In the same fashion, average total tumors were 4.37 times as frequent in males, and 2.28 times as frequent in the females as in the grouped positive controls (see Table 6, Appendix C). The thyroids were not examined. The dose by gastric intubation was 215 mg/kg of body weight daily for the first 28 weeks of life, and 646 ppm in the diet thereafter. The study was well conducted; adequate negative and positive controls were used.

In 1972, Ulland et al reported that in rats administered either 175 or 350 ppm ETU in the diet, there was an increased incidence of thyroid carcinoma. Six of 56 (10.7%) low-dose-treated rats developed

carcinoma of the thyroid (10.7%). At the high dose 25 of 56 (44.6%) developed carcinoma of the thyroid. No thyroid carcinomas were noted in 64 matched controls (historical controls incidence, 3/452).

Graham et al (1973) reported increased thyroid carcinoma in rats after a 1-year administration of ETU. This was followed by a later report in 1975 on the results of 2 years of administration of ETU in the diet. At 500 ppm ETU in the diet, 62 of 70 (89%) developed thyroid carcinoma. Two of 72 (3%) control rats developed thyroid carcinomas. Both of the above investigations were carefully conducted, utilizing adequate controls and providing in-depth pathological evaluations.

Gak et al (1976) reported carcinogenicity in a lifetime study in rats, but not in hamsters, at 200 ppm ETU in the diet. At the lower level of 60 ppm of ETU in the diet, ETU was also carcinogenic for male rats but not females. This study appears well designed and well conducted, but tumor rates are given in percent only rather than in actual numbers, making adequate statistical evaluation impossible. Survival rates were not stated.

A dose-response graph of the carcinogenicity of ETU in rats is shown in Figure 1. It can be seen that while the data from the Graham and Ulland studies fit quite well on a straight line, dose-response relationship, the strain of rat used by Gak et al (1976) was more susceptible to the carcinogenic effect of ETU than the strain used by Graham et al (1975) and Ulland et al (1972).

The following table summarizes data from six studies of the carcinogenicity of ETU. The lowest dose of ETU which has produced a neoplasmic change is given in each case so as to compare species,

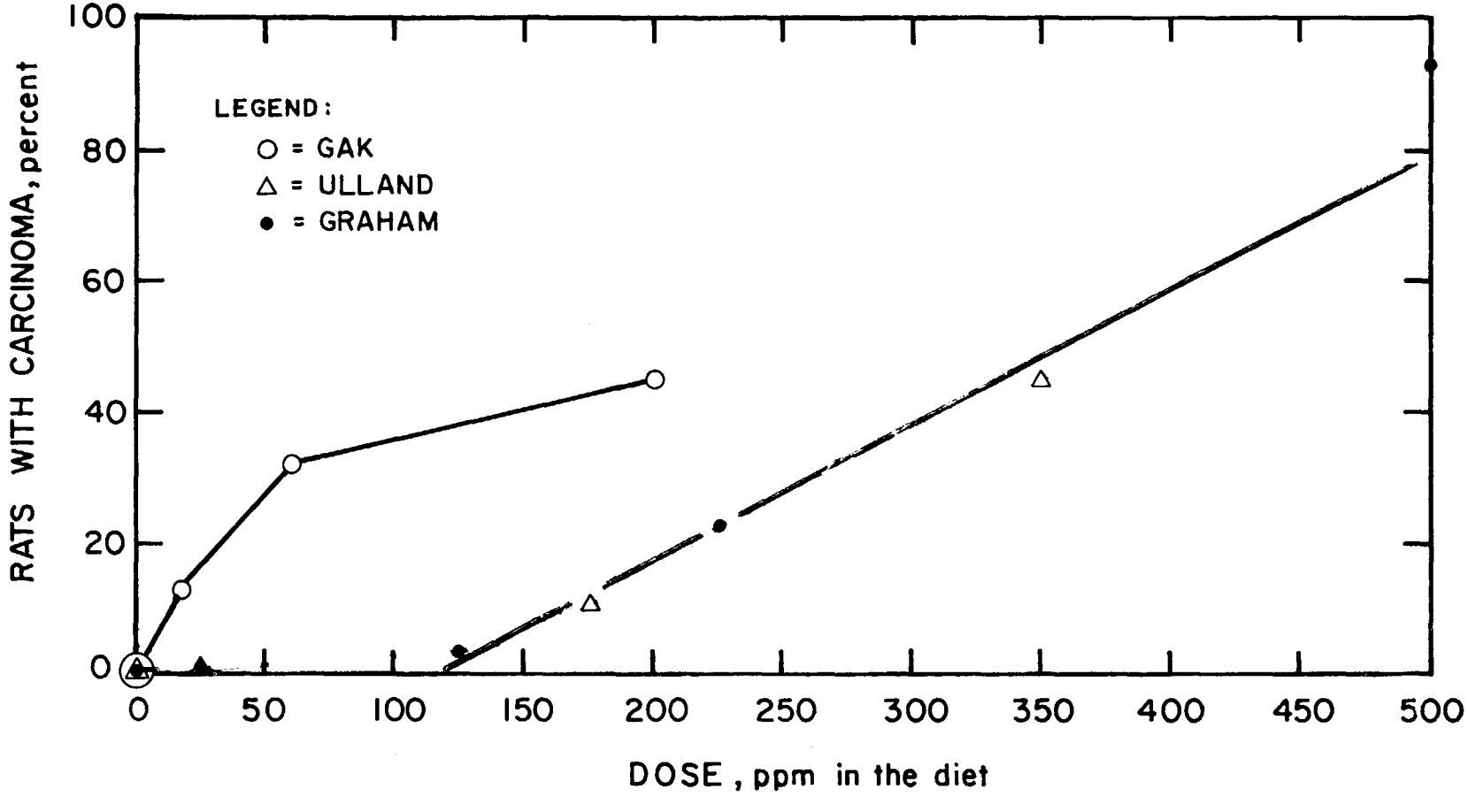

Figure 1. Ethylene Thiourea dose / carcinoma, a summary of three investigations.

strain, and the type of tumor which resulted. In each of the studies where higher doses were also used, the higher doses resulted in further increased incidences of neoplasia.

LOWEST DOSE
OF ETHYLENE THIOUREA
WHICH PRODUCED
NEOPLASTIC CHANGE

Investigators	Dose ppm	Dose mg/kg	Types of Tumors and Incidence		Species and Strains
			Treated	Controls	
Innes et al (1969)	(7-28 days)		Lymphomas		Mouse (C57 B1/6 x C3H Hybrid), (C57B1/6 x AKR Hybrid)
	--	215	7/24	5/172	
	(28 days-death)				
	646	62*	Hepatomas		
			59/68	14/338	
Graham et al (1973) (1 yr)	500	40*	Thyroid carcinoma		rat (Charles River, Sprague-Dawley)
			15/25	0/22	
(1975) (2 yr)	250	20*	Thyroid carcinoma		rat (Charles River, Sprague-Dawley)
			16/32	2/32	
Ulland et al (1975)	350	28*	Thyroid carcinoma		rat (Charles River, Sprague-Dawley)
			6/52	0/64	
Gak et al (1976)	60	5*	Thyroid carcinoma		rat (Unspecified strain)
			(actual number not given)		
			44.4%	3.2%	
Freudenthal et al (1977)	625	15*	Thyroid adenoma		rat (Charles River Spraque-Dawley)
			7/20	0/20	

*Calculated from body weight and average food consumption.

Average doses are given in both ppm and in mg/kg to allow for direct comparisons among the various studies. The assumptions in these calculations were that the purity of ETU used by the various investigators was the same, ETU was uniformly distributed in the food, and food consumption estimates were accurate.

D. Mutagenicity and Cell Transformation

It has been established for a wide range of chemical classes that most animal carcinogens are also mutagens or produce mutagenic metabolites under appropriate conditions (McCann et al, 1975; Miller and Miller, 1971). Consequently, short-term tests, both in-vitro and in-vivo, have become a generally accepted indication of potential carcinogenic hazard.

The most frequently utilized test system for mutagenesis is that of Ames et al (1973) in which the substance to be tested is added to an innoculum of Salmonella typhimurium and grown on a special culture medium. The percent of reversion of an enzyme deficient strain to the wild strain of S. typhimurium is proportional to the extent of alteration in DNA that the substance produces. The more reversions that occur, the more mutagenic that substance is said to be (Ames, 1973).

ETU produced statistically significant numbers of revertant colonies when tested with the S. typhimurium TA-1535 strain. It produced no revertants in tests with TA 1538-8 and the E. coli BP-2 series. This included metabolic activation tests with S-9 liver enzymes with these latter two strains. In vitro cytogenetic tests using Chinese hamster cells and chromosome anomaly tests on rat bone marrow cells gave negative results. Mouse dominant-lethal assay (DLA) tests were also negative (Shirasu, 1975).

In an investigation by Sram (1975), single or repeated doses of ETU produced negative results in the dominant-lethal assay test. He reported the frequency of chromosomal aberrations in the bone marrow

"increased," and this effect was dependent on dose. Seiler (1975) reported that when ETU was fed to mice together with sodium nitrite, the incidence of micronucleated red blood cells in the bone marrow was "greatly increased." Seiler (1974) also reported mutagenic activity for ETU in the S. typhimurium G-46 strain. There was a 2.5 fold increase in mutation frequency at "intermediate" concentrations of ETU. Higher concentrations of ETU killed the bacteria.

Schuepbach and Hummler (1976) reported ETU to be a mutagen. There was a rise in the number of colonies of revertant strains with the "repair-deficient" strain TA-1503. The reversion rate was 7.1 at 20, 9.1 at 40, 11.7 at 80, and 11.1 at 200 mg/plate. Reversions with the G-46 strain were not significantly different from controls at 20, 40, 80, or 200 mg/plate. No induction of revertants was observed utilizing as the test system the "frameshift" mutants TA-1531, TA-1532, or TA-1964. Using a host-mediated assay, the reversion frequency was 2.4 times greater than the controls in strain TA-1503. Other strains were negative. No mutation occurred at doses lower than 20 mg/plate. In contrast to the results of Seiler (1975) reported on above, Schuepback and Hummler found no micronucleated RBC's in Swiss albino mice given 2 doses (in 24 hours) of 25, 700, 1,850, or 6,000 mg/kg of ETU. The DLA test at 500, 1,000, and 3,500 mg/kg resulted in a non-dose related slight reduction in fertility rate.

LeBrecque and Gauck (1963) reared Musca domestica larva to adulthood and observed any abnormalities caused by ETU. The toxic concentration was reported to 1% of the diet. Sterilization of the

larva was found at 1%, 0.5%, 0.25%, and 0.1% ETU in the diet. Sterilization was inconsistent at 0.1%.

The above studies indicate ETU is mutagenic in at least two typhimurium strains, and ETU interacts with DNA to produce inheritable changes in specialized bacterial cell lines.

E. Teratogenicity

Ruddick and Khera (1975) reported that a single oral administration of 240 mg/kg of ETU to rats on one of gestation days 10 to 21 produced visceral anomalies involving the nervous, urogenital, and ocular systems, as well as osseous anomalies affecting both the axial and appendicular skeletons. The type of anomaly found was dependent upon the day of treatment, since organogenesis impairment is related to the stage of ontogenesis at the time of treatment. Khera (1973) proposed that the mode of action of ETU on the embryo is unique in that anomalies that ordinarily are mutually exclusive are often found simultaneously, for example hydrocephalus and exencephaly. The concomitant production of these 2 anomalies could not be due to a simple disturbance of the organ anlage because day of treatment was after the stage of differentiation (on the average). Even after day 16 (which marks the end of organogenesis), hydroptic defects were observed, including hydrocephaly, hydranencephaly, and subcutaneous edema. Khera suggested this was due to altered vascular permeability, especially along the borders of the ventricular cavities of the CNS.

A recent report from du Pont's Haskell Laboratories (Stula and Kraus, 1977) states "marked teratogenic effects were demonstrated with ETU." In this study, 60 mg of ETU in dimethyl sulfoxide (DMSO) per kg of body weight was applied to the skin of the dam at day 12 or 13 of gestation. This produced malformations in 100% of 73 fetuses without any observable significant effects on the dam. The dermal dose (60 mg/kg) used was 1/45th of the approximate lethal dose of the dam. Control dams treated with DMSO or water had embryos which had no deformities. However, in a previous experiment, one DMSO-treated dam had one embryo with an encephalocele.

The teratogenic effect of ETU is primarily dystectic (ie along the neural canal). In an extensive and detailed report by Khera and Tryphonas (1977), dams dosed at 30 mg/kg on day 15 of gestation had offspring characterized by progressive neural tube and ependymal necrosis, leading to enlargement of the entire ventricular system. In this carefully conducted study, it was also shown that the resulting anomalies were not dysgeneic, and offspring of the second generation were normal in size and structure.

Additional studies demonstrating the teratogenicity of ETU in the rat are reported in the literature (Khera, 1973; Shirasu, 1975; Stula, 1977; Teramoto, 1975). Dose levels used and resultant abnormalities were similar to those described above.

V. EVALUATION AND CONCLUSIONS

The investigations that demonstrate ETU is carcinogenic were presented and discussed previously in Section IV-C of this review. It was first shown to induce hepatomas and lymphomas in mice (Innes et al, 1969). It was next shown to produce thyroid adenomas and carcinomas in the rat in a dose-dependent fashion by two independent investigators (Ulland et al, 1972; Graham et al, 1975). A higher incidence of tumors was then reported in a more susceptible strain of rat (Gak et al, 1976). Lastly, adenomas of the thyroid were shown to occur following 90 days exposure in rats (Freudenthal et al, 1977). Since ETU is a potent goitrogen, it had been suspected as a thyroid carcinogen prior to specific testing, since other goitrogens such as propyl thiouracil had been shown to be carcinogenic (IARC, 1976).

In the past, the carcinogenicity of industrial chemicals was discovered only by finding tumors in the workmen handling these chemicals. Presently, agencies of the Federal government are determining carcinogenic hazard on the basis of results established in experimental animals (Federal Register #192, 1977).

From the foregoing evidence, NIOSH concludes that in experimental animals, ethylene thiourea (ETU) is a carcinogen and a teratogen. Exposure to ETU as an isolated chemical presently occurs only in specific occupations, ie ETU synthesis, mixing operations, and laboratory experimentation. The major use of ETU is in the synthetic rubber industry.

Ethylene thiourea is considered by NIOSH to present a potential carcinogenic and teratogenic hazard to U.S. workers. Control measures are therefore warranted for protecting against this hazard.

Thyroid cancer is a relatively rare disease (Anon, NCI, 1976). It accounts for only 0.4% of all the cancer deaths and for only 6 deaths per million of the population each year. Approximately 1,150 deaths occurred in the U.S. in 1976 due to this neoplasm. About 8,100 new cases were diagnosed during the same year (Anon, NCI 1976). It occurs twice as frequently in females as males, and more frequently in whites than blacks. However, a much more frequent condition, termed multinodular goiter, occurs in the general population. As many as 4% of normal people are found to have cancer-in-situ of the thyroid (De Groot and Stanbury, 1975).

For the 23 years from 1947 to 1970, an NCI survey (NCI, 1976) has revealed an increase in incidence of thyroid cancer of only 2.4 to 3.9/100,000 population. By comparison, the incidence is as high as 33% in individuals who have received head and neck radiation for non-malignant conditions (Farus, 1976). While the combined effect of irradiation and antithyroidals on tumor incidence in humans is unknown, Doniach (1970) reported X-rays plus antithyroidals in rats produced greater numbers of thyroid tumors with a higher incidence of malignancy than either treatment alone. Thus, although radiation is no longer considered a good method of treating non-malignant conditions of the head and neck, NIOSH draws attention to the potential increased risk of carcinogenesis for workers who have received such therapeutic radiation in the past and who also are potentially exposed to ETU. Workers

previously exposed to radiation should be counseled on the possibility of the combined effect of radiation and ETU exposure presenting an increased health hazard.

The only epidemiology study of workers exposed to ETU failed to demonstrate that ETU produces cancer in humans (Smith, 1976). A causal relationship between ETU and tumors in the U.S. working environment would be difficult to establish epidemiologically. This is due both from the often transient employment in compounding, mixing and milling operations, and from the simultaneous exposure to a variety of different chemicals during employment. This is true even in the manufacture of ETU, since manufacture tends to be in batch operations, often shifting from making one chemical to making another.

Present lack of evidence in the worker population cannot be considered as an indication that ETU is without carcinogenic effect in the human. ETU has not been used over a sufficient length of time to establish its carcinogenic effect in the worker. In addition, cancer of the thyroid may not be the only site of carcinogenic effect of ETU exposure, since one organ site of oncogenicity in one species may manifest itself at a different site in another species. For example, hepatomas and lymphomas are reported in mice (Innes, 1969), while thyroid carcinoma is reported in rats.

At the present time, there is no known safe level of exposure to ETU with respect to carcinogenic or teratogenic potential.