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SPECIAL OCCUPATIONAL HAZARD REVIEW with CONTROL RECOMMENDATIONS
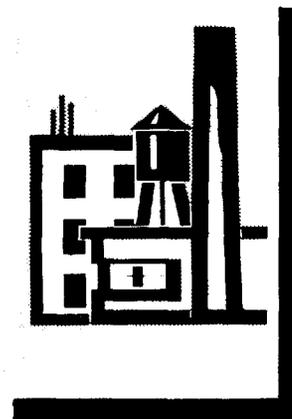
for ETHYLENE THIOUREA

SPECIAL HAZARD REVIEW

WITH

CONTROL RECOMMENDATIONS

FOR

ETHYLENE THIOUREA

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DISCLAIMER

Mention of company names or products does not constitute endorse-
ment by the National Institute for Occupational Safety and Health.

PREFACE

The Occupational Safety and Health Act of 1970 emphasizes the need for standards to protect the health and safety of workers exposed to an ever increasing number of potential hazards in their workplace. Consequently, the National Institute for Occupational Safety and Health (NIOSH) has implemented a program to evaluate the adverse effects of widely used chemical and physical agents. This program includes the development of Special Hazard Reviews which serve to support and complement the other major criteria documentation activities of the Institute.

The purpose of the Special Hazard Review is to assist employers in protecting the health and well-being of their employees.

The design of a Special Hazard Review is to analyze and document, from a health standpoint, the problems associated with a given industrial chemical, process, or physical agent, and to recommend the implementation of engineering controls and work practices to correct these problems.

Special Hazard Reviews are intermediate in scope to the more comprehensive NIOSH Criteria Documents and the briefer NIOSH Current Intelligence Bulletins. Generally, Special Hazard Reviews will concern those hazards associated with cancer or reproductive effects.

Dissemination of Special Hazard Reviews may be accomplished through appropriate trade associations, unions, industries, and members of the scientific community.
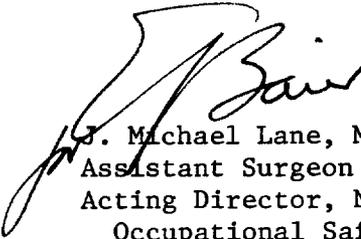
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ACKNOWLEDGEMENTS

SPECIAL HAZARD REVIEW
ETHYLENE THIOUREA
(ETU)

CONTENTS

I. INTRODUCTION

On April 11, 1978, NIOSH issued a Current Intelligence Bulletin
(CIB) which called attention to the carcinogenic and teratogenic
potential of ethylene thiourea (ETU) in the workplace (Ethylene
Thiourea, Current Intelligence Bulletin #22). While this bulletin
alerted industry to known ETU hazards, the following Special Hazard
Review presents a further report of the current occupational exposure
to ethylene thiourea, and a more detailed and extensive appraisal of
its mutagenic, teratogenic, and carcinogenic potential hazard in the
workplace. This review was compiled so that a reliable source of
information can be readily available to management and labor to assess
the preventive measures available, and form a basis for the development
of those better work practices needed to adequately protect the worker
from exposure to ETU.

ETU is an organic compound chemically known as 2-
imidazolidinethione. Its primary use is as an accelerator for
vulcanization of elastomers (The Condensed Chemical Dictionary, 1977;
Merck Index, 1977; Hill, 1977). It has also been used for a variety of
other applications including: a clearing agent in metallic
electroplating baths, and an intermediate in dyes, synthetic resins,
antioxidants, and pharmaceuticals (Merck Index, 1977).

The effects of prolonged exposure to ETU in experimental animals
are: hypothyroidism and neoplastic transformation of body tissues
(Graham et al, 1973; Gak et al, 1977).

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ETU is also an in-vitro and in-vivo breakdown product of the ethylenebisdithiocarbamate type of fungicides (Bontoyan, 1973, 1975; Newsome et al, 1975). Mainly on the basis of the potential pollution of the environment by ETU from these fungicides, EPA has issued a Rebuttable Presumption Against Registration (RPAR) for ethylene bisdithiocarbamates (Federal Register, #154, 1977).

In the Russian publication "Harmful Substances in Industry," reference is made to ETU as the toxic breakdown product from the ethylenebisdithiocarbamate fungicides as the basis of hazard therefrom (Lazarew, 1978).

The Food and Drug Administration has revoked use of neoprene and rubber products which contain ETU that may come in contact with drugs, cosmetics or medical devices (Federal Register, 1973, 1974).

The World Health Organization (WHO) has not established a permanent Allowable Daily Intake (ADI) for ETU, but has set guidelines for residues (on crops) which vary from 0.01 to 0.1 ppm (WHO, Personal Communications, March, 1978; Ethylene Thiourea, IUPAC, 1977).

ETU was evaluated by the International Agency for Research on Cancer (Ethylene Thiourea, IARC Monograph, Volume 7, 1974), and on the evidence presented, ETU was designated "carcinogenic."

No Federal regulations limiting industrial exposure of workers to ETU have been established in the United States, nor by any foreign government reporting their regulations to the World Health Organization (Permissible Levels of Toxic Substances, 1977).

ETU is listed under the heading of "Carcinogenic Substances and Agents Whose Use Presents Significant Potential Hazard," by the

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International Labour Office of the World Health Organization (Occupational Cancer--Prevention and Control, 1977).

II. CHEMICAL AND PHYSICAL PROPERTIES

At room temperature pure ETU is a colorless, odorless crystalline solid with a bitter taste (Kare and Muller, 1965). Some of the properties of ETU are listed in Table I.

Table I

Properties of Ethylene Thiourea

MW	102.17
Composition	C 3 N 2 S H 6
Structural formula	

$$\begin{array}{ccc} HCH \text{---} NH & & \\ | & \diagdown & \\ | & C{=}S & \rightleftharpoons \\ | & \diagup & \\ HCH \text{---} NH & & \end{array} \qquad \begin{array}{ccc} HCH \text{---} N & & \\ | & \diagdown\diagdown & \\ | & C{-}SH \\ | & \diagup & \\ HCH \text{---} NH & & \end{array}$$
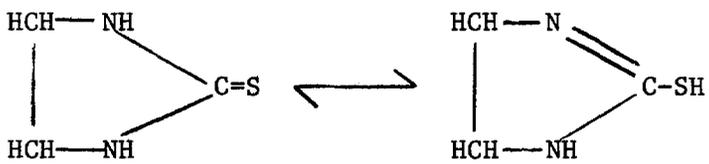
Physical state	colorless short needle crystals or prisms
Melting point	203C
Vapor pressure	unknown
Flash point	252C
Water solubility	2% at 30C, 9% at 60C
	44% at 90C

(Weast, 1977; The Condensed Chemical Dictionary, 1977; Hill, 1975)

ETU is stable in air or in solution over a wide range of temperatures and pH levels (Cruickshank and Jarrow, 1973). Ultraviolet

radiation breaks down ETU to ethylene urea if a sensitizer, such as acetone or riboflavin, is added.

III. CHARACTERISTICS OF EXPOSURE

A. Manufacture

Hofman first synthesized ETU from ethylene diamine and carbon disulfide while attempting to prepare a mustard oil (Hofman, 1872). Most of the methods now used for the synthesis of ETU are variations of this, but Matolcsy (1968) demonstrated that it could also be synthesized from KCN and CS2.

In industry, ethylene thiourea is synthesized from ethylene diamine and carbon disulfide (Merck Index, 1976). Most of the production results in colorless to light green to yellow crystals with a light amine odor. This crystalline form melts at approximately 197C (The Condensed Chemical Dictionary, 1977). These crystals are crushed into a very fine powder in order to promote good dispersion into the elastomer. This flour-like dust tends to stick to the hands or on agitation becomes dispersed into the air (E.I. du Pont, 1972; Health Hazard Evaluation, 1977).

At least three major companies produce a safer type of formulation of ETU for use in the neoprene fabricating industry. The manufacturers intercalate finely divided ETU powder inside a matrix of an elastomer or a wax which is compatible with the final product. This "encapsulated" form of ETU is then used as the accelerator additive for vulcanization. In this form, ETU is least likely to become dispersed into the air that is respired by employees in facilities fabricating chloroprene products.

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B. Production Volume

Production estimates of ETU are not available, the information being considered proprietary in nature by all companies (Synthetic Organic Chemicals, 1976). ETU production is closely tied to chloroprene (2-chlorobutadiene) polymer production and vulcanization. An estimate of the total production of ETU can thus be made from the production of chloroprene. A NIOSH criteria document has been prepared on occupational exposure to chloroprene (Criteria Document, 1977). Chloroprene is the monomer used to produce both chloroprene polymer latexes which are soft, as well as "dry" rubbers which are more resilient. Chloroprene polymer production is estimated at 440 million pounds per year (Chemical Profile, 1976). Inasmuch as ETU is reported to be added to chloroprene polymer at a rate of approximately one percent (1%) (E.I. du Pont, 1972), the best estimate of the production of ETU on this basis is 4.4 million pounds per year. Such an estimate does not include use in the acrylate base elastomers or in other uses, such as in electroplating, intermediates in the manufacture of other compounds, or as a contaminant found in fungicides (IARC, 1974; Bontoyan et al, 1975; Merck Index, 1977; The Condensed Chemical Dictionary, 1977).

Chloroprene polymer use has increased 5-6% per year from 1950-1974 (Criteria Document, 1977). The future growth has been estimated at 2.4% per year (Chemical Profile, 1976). The production of chloroprene polymers has been diminished by the use of plastics, especially in the wire and cable end-use area. Chloroprene polymer demand is dependent largely on the auto and construction industries, and, therefore,

chloroprene polymer production is correlated with the general economy (Chemical Profile, 1976). The U.S. International Trade Commission lists two major producers of ETU (Synthetic Organic Chemicals, 1976): E.I. du Pont de Nemours and Co., Inc., and Fike Chemicals, Inc. Current information indicates ETU is distributed and available from most major chemical companies in small as well as bulk quantities (personal communications, 15 chemical companies, 1977).

C. Uses

Ethylene thiourea is used extensively as an accelerator in the vulcanization of various elastomers, including polychloroprene. Neoprene is E.I. du Pont's trade name for chloroprene polymer (polychloroprene). Chloroprene polymer, unlike natural rubber or most synthetic rubbers, is capable of being vulcanized or "cured" without the addition of any curing agents or accelerators, but the state of cure is usually unacceptable for most applications, and the rate of cure is too slow to be economically feasible (Neal, 1950). Because of this weakness in ability to vulcanize readily without additives, metal oxides, usually zinc oxide and manganese oxide, are employed as curing agents, and for most applications additional accelerators are desirable (Neal, 1950). The metal oxides furnish the free radicals for inducing the cross-linking of the monomer molecules. ETU initiates the free radical production at a more rapid rate. Neal (1950) discovered that in addition to producing a more rapid vulcanization, ETU was effective in producing "good age resistance, high elastic efficiency, and remarkable resistance to permanent deformation under conditions of

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compression." To date, no well accepted substitute for ETU has been found (personal communications, du Pont, Wyrough and Loser, St. Clair Rubber Co., 1977, 1978).

ETU can be used in nearly every type of polychloroprene formulation compound when a rapid rate of cure is desired. It can be used alone or in combination with other accelerators and curing agents. Variation in rate of addition can produce the most desired balance of properties in the vulcanizate. In general, modulus, resilience, and compression-set resistance all increase in proportion to the amount of ETU added up to "several" parts per hundred parts of elastomer. At the same time, processing safety, as indicated by the Mooney scorch test, (E.I. du Pont, 1972) decreases as ETU concentration increases. The various types of chloroprene polymer are somewhat different in their ETU requirements. In certain types, it is especially important to include a modifying secondary accelerator, such as thiuram, to produce the desired properties (E.I. du Pont, 1972; Bikales, 1965)

In thiacril rubbers, ETU meets the criteria of good curing performance and good storage characteristics, as well as adding the advantages of being non-adhesive to the mold and non-corrosive (E.I. du Pont, 1972).

Most uses other than as an accelerator in vulcanization are regarded by industry as proprietary information, but Merck Index (1977) lists its use in the electroplating of metals, and as an intermediate in the manufacture of dyes, synthetic resins, antioxidants, and pharmaceuticals.

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D.   Extent of Occupational Exposure

Based   on   a   national   survey   conducted from 1972 to 1974, NIOSH estimated that approximately 17,000 employees   in   24   different occupations are potentially exposed to ETU in the working environment (see Appendix C). The preliminary report of a Health Hazard Evaluation of ETU in a chloroprene fabrication plant estimates the major exposure routes to be inhalation and skin contact (Salisbury, 1978). This preliminary report also establishes that occupational exposure is less likely to occur when an "encapsulated" formulation of ETU is utilized rather than ETU powder.

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 radio-active 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

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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).

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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

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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

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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,

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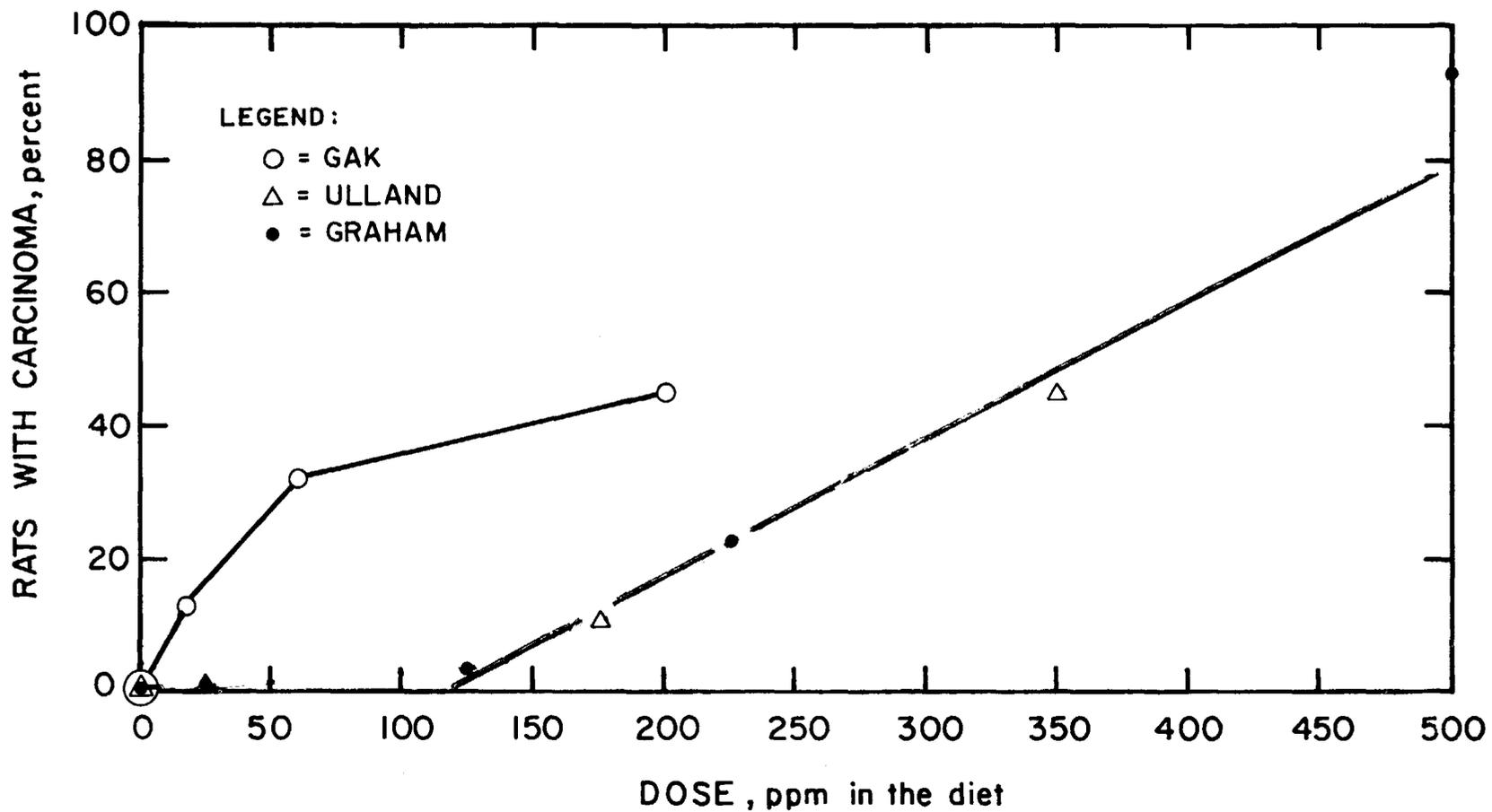
Figure 1. Ethylene Thiourea dose / carcinoma, a summary of three investigations.

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	mg/kg	Types of Tumors and Incidence		Species and Strains
			Treated	Controls	
Innes et al (1969)	(7-28 days) --	215	Lymphomas 7/24	5/172	Mouse (C57 Bl/6 x C3H Hybrid),
	(28 days-death) 646	62*	Hepatomas 59/68	14/338	(C57Bl/6 x AKR Hybrid)
Graham et al (1973) (1 yr)	500	40*	Thyroid carcinoma 15/25	0/22	rat (Charles River, Sprague-Dawley)
(1975) (2 yr)	250	20*	Thyroid carcinoma 16/32	2/32	rat (Charles River, Sprague-Dawley)
Ulland et al (1975)	350	28*	Thyroid carcinoma 6/52	0/64	rat (Charles River, Sprague-Dawley)
Gak et al (1976)	60	5*	Thyroid carcinoma (actual number not given) 44.4%	3.2%	rat (Unspecified strain)
Freudenthal et al (1977)	625	15*	Thyroid adenoma 7/20	0/20	rat (Charles River Spraque-Dawley)

*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

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"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.

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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.

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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

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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.

VI. RECOMMENDATIONS

The major NIOSH recommendation for the synthetic rubber industry is complete conversion from the use of the powdered form of ETU to the "encapsulated" form (see Section III-A and D of this review). In this "encapsulated" formulation, ETU is least likely to escape into the environment and subsequently be taken into the body of the worker. The efficacy of this recommendation is apparent from the preliminary report of the NIOSH Health Hazard Evaluation on ETU (Salisbury, 1977). While approximately 100 rubber fabrication plants are presently utilizing "encapsulated" ETU, it is estimated that approximately 100 to 150 companies have not made this change in procedure (personal correspondence, Wyrough & Loser, Inc. 1977).

Further recommendations include proper labeling of all non-encapsulated ETU as carcinogenic and teratogenic, adequately informing employees of the potential hazard, and proper use of sanitation practices in the workplace. Compliance with all sections of these recommendations should at a minimum reduce the risk of ETU-induced cancer and prevent other adverse effects of occupational exposure to ETU. "Occupational exposure to ETU" is defined as work in any place in which ETU is produced, stored, used, packaged, or distributed. Work in a place utilizing "encapsulated" ETU only is not considered "occupational exposure". Detailed recommendations are as follows.

Informing Employees of Hazards from ETU

At the beginning of their employment in an ETU area, workers should be informed of the hazards, relevant symptoms of overexposure, appropriate emergency procedures, and proper conditions and precautions for safety. The information should be kept on file and should be readily accessible to the worker at all places of employment where occupational exposure to ETU is likely.

Employers should institute a continuing educational program to ensure that all workers have current knowledge of job hazards, proper maintenance procedures, and cleanup methods, and that they know how to use respiratory protective equipment and protective clothing correctly. Employees should be informed of the possible additive effects from taking antithyroidal medication. Employees breast-feeding an infant should be informed that ETU fed to experimental animals was found in the milk of those animals.

Personnel who must move supplies of ETU, clean up spills, and repair leaks should be properly trained in such procedures and adequately protected against the attendant hazards.

Labeling and Posting

(a) Labeling

Containers of ETU should bear the following label in addition to, or in combination with, labels required by other statutes, regulations, or ordinances:

ETHYLENE THIOUREA

WARNING

MAY CAUSE CANCER OR BIRTH DEFECTS

IF INHALED OR SWALLOWED

AVOID SKIN CONTACT

No smoking.

Avoid breathing dust or spray mist.

Avoid contact with eyes, skin, and clothing.

Wash hands and face thoroughly before eating (a bitter taste

   indicates residual ETU on hands).

Wear long-sleeved work clothes.

Shower or bathe and change into clean clothing after work.

(b) Posting

The following sign should be posted in a readily visible location
at or near entrances to manufacturing and formulating areas containing
ETU and at other areas in which there is a risk of exposure:

ETHYLENE THIOUREA

CAUTION!

MAY CAUSE CANCER OR BIRTH DEFECTS

HARMFUL IF INHALED OR SWALLOWED

NO SMOKING

AVOID SKIN CONTACT

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Avoid breathing dust or spray mist.

Avoid contact with eyes, skin, and clothing.

Wash hands and face thoroughly before eating.

Wear long-sleeved work clothes.

Shower or bathe and change into clean clothing after work.

Warning signs should be printed in English and in the predominant language of non-English-reading employees, if any, unless employers use equally effective means to ensure that non-English-reading employees know the hazards associated with ETU and the areas in which there is exposure to ETU. Employers should ensure that employees having difficulty reading signs also know these hazards and the locations of these areas.

Engineering Controls

Engineering controls, such as process enclosure or local exhaust ventilation, should be used whenever possible to prevent airborne concentrations of ETU or contact with the skin. Ventilation systems should be designed to prevent the accumulation or recirculation of ETU in the workplace and to remove ETU effectively from the breathing zones of employees. Ventilation systems should undergo regular preventive maintenance and cleaning to ensure maximum effectiveness, and this effectiveness should be verified by monthly airflow measurements. In addition, environmental monitoring is recommended to determine the effectiveness of engineering controls. The recommended sampling and analytical method described in Appendix A, if used, will detect

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environmental levels of ETU as low as 30 micrograms per cubic meter of air.

General Medical Recommendations

Those workers with a history of thyroid disease, pulmonary disease, cardiovascular disease, or renal disease, and those using antithyroid drugs should be counseled about working in jobs involving exposure to ETU. Workers should be advised that a review of the available scientific data warrants consideration of possible effects of ETU on reproduction, and that information based on experimental animal studies indicates the possible induction of severe defects in the developing fetus, especially in the central nervous system. Female workers of reproductive age should be advised of the potential for malformation of the developing fetus. Pregnant workers or workers breast-feeding an infant should not be exposed to ETU under any circumstances.

For employees assigned to an area where ETU is used, medical examinations should be made available on a yearly basis or at some other interval determined by the responsible physician. Special attention should be paid to the function of the thyroid during the medical evaluation of the health status of the worker.

At the time of the preplacement examination, it is recommended that a pre-exposure baseline be determined for thyroid-stimulating hormone, thyroxine and triiodothyronine.

Sanitation Practices

Employees working in areas where ETU is manufactured, processed, handled, or stored should wash their hands before eating, drinking, smoking, or using restroom facilities during the work shift.

No food or beverages should be stored, prepared, or consumed in areas when ETU is manufactured, processed, handled, or stored.

Contaminated clothing should be removed before entering areas where food or beverages are consumed.

Smoking should be prohibited in areas where ETU is manufactured, processed, handled, or stored in unsealed containers.

Employees should shower or bathe and change clothing after the workday if any possible dermal exposure could have occurred.

Disposal

Work areas, fixtures, equipment, etc, contaminated by ETU spills should be cleaned promptly. ETU powder on floors should be blotted with absorbing clay which, in turn, may be removed with a sweeping compound. An alkaline solution of hypochlorite will oxidize ETU into ethylene urea (Hylin, 1973). Thus, a one to ten dilution of commercially available 5% hypochlorite solutions may be used to mop up areas contaminated with ETU.

In work areas where ETU is used in powder form, proper exhaust ventilation should be used. In many secondary uses of ETU, the dust hazard may be eliminated by the substitution of sheets or pellets of ETU (dispersed in a plastic material) for powdered ETU when it is used as a direct additive. These specialty forms of ETU ("encapsulated"

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pellets or sheets) are available for various elastomer products made by rubber companies in the U.S. Special formulations are available on a custom-made basis from several companies. Whenever possible, these dust-free "encapsulated" formulations should be utilized.

Personal Protective Equipment and Clothing

(a) Protective Clothing

Any employee whose work involves likely exposure of the skin to ETU should wear full-body coveralls or the equivalent, impervious gloves, ie, highly resistant to the penetration of ETU, impervious footwear, and, when there is danger of ETU coming in contact with the eyes, goggles or a face shield. Any employee engaged in mixing operations where use of powdered ETU is required, should be provided with the following protective clothing and equipment: goggles, full-body coveralls, impervious footwear, and a protective head covering.

(b) Respiratory Protection

Engineering controls should be used to maintain airborne ETU concentrations at the lowest possible level. Respiratory protective equipment should be used in the following circumstances: during the time necessary to install or test the required engineering controls, for operations such as nonroutine maintainance and repair activities, and during emergencies when concentrations of airborne ETU may exceed the lowest detectable level of ETU (30 micrograms/cubic meter) in air.

In all cases where respiratory protective equipment is required, only supplied - air respirators should be used.

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ERRATA NOTICE

Special Hazard Review with Control Recommendations
for
ETHYLENE THIOUREA
DHEW(NIOSH) Publication No. 79-109

Substitute the attached Appendix A for the Appendix A
in the above mentioned book.

To be inserted in the above mentioned publication.

APPENDIX A

SAMPLING AND ANALYTICAL METHODS

There are a number of analytical techniques available in the literature which may be used to detect ETU in air, solutions, and solids. This multiplicity of methods has been largely due to the need of many investigators to detect minute amounts of ETU as contaminants or metabolic products of the ethylenebisdithiocarbamate fungicides.

The two main categories of detection have involved gas liquid chromatography (GLC), or wet chemistry (WC) methods, with GLC methods generally having greater sensitivity, specificity, and accuracy. However, most are best adapted for research rather than routine analysis.

The detectors utilized thus far in GLC have included electron capture (ECD), thermionic (TID), and flame photometric (FPD) detectors. While smaller sized samples may be used with the ECD, derivatization of the sample must usually be performed before detection. This step can be eliminated in the FPD method, but other FPD methods reported utilize derivatization which, of course, increases specificity. While selective GLC methods are more often used in research on ETU where confirmation of the identity of ETU is essential, it may also be used in routine analyses of air and solution samples as well. Solid samples nearly always require additional procedures.

In nearly all cases, the stepwise procedure for GLC analyses involves: (1) extraction, (2) cleanup, (3) derivatization, and (4) determination. Extraction is usually performed with methanol or ethanol but mixtures using acetone, chloroform, trichloroacetic acid and sodium ascorbate have also been used. Cleanup involves eliminating interfering substances. This is especially necessary in biological samples containing ETU. Cleanup is most efficiently done by separation of a derivative of ETU from an aqueous sample solution. Column chromatography or solvent partitioning may also be used (Onley and Yip, 1971).

In derivatization, the thio group is used for alkylation. This allows for the primary distinction among classes of chemicals similar to ETU. Various derivatives have been championed, each with various advantages. The final determination of the derivative is established by the partition coefficient along selected distinctive column packings.

Additional techniques for ETU analysis include: (1) thin layer chromatography (Vonk, 1970, Blazquez, 1973, Olney and Yip, 1971, and Engst, 1974)--this method generally has a lower detection limit of 20 ppb--; (2) polography (Engst, 1974)--here the nitroso derivative is determined after alumina column cleanup--; (3) Radioactive derivatization (Nash, unpublished); (4) Spectographic (NIOSH standard method, Palassi, 1977). This simple, as yet unpublished spectophotometric method for the determination of ETU is especially suited to measurement of air samples. It has the combined advantages of direct reading, good sensitivity, and adequate specificity (all thio

groups). It is relatively fast and does not require expensive instrumentation. The NIOSH-verified method of analysis of ETU is presented below:

ETHYLENE THIOUREA

(2 Imidazoladinethione)

Measurements Support Branch

Analytical Method

Analyte:	Ethylene thiourea	Method No.:	P & CAM 281
Matrix:	Air	Range:	$0.03-1.5$ mg/m^3 for 100 1 sample
Procedure:	Filter collection, extraction, complexation, spectrophotometry	Precision:	3% (Analytical)

1. Synopsis

Ethylene thiourea is collected from air on a PVC or cellulose ester membrane filter. The filter is extracted with distilled water. Pentacyanoammine-ferrate reagent is added to the extract to form a colored coordination

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complex. The absorbance of the solution is measured spectrophoto-
metrically at 590 nm, and the concentration of ethylene thiourea is
determined from a calibration curve.

2. Working Range, Sensitivity, and Detection Limit

2.1 The working range for this method is 0.03-1.5 mg/m^3 in 100 1 sample.
     The entire linear working range has not been determined.

2.2 The sensitivity is 0.006 absorbance units/µg, as determined from
     the slope of several calibration curves.

2.3 The detection limit is 0.75 µg/sample or 0.0075 mg/m^3 in 100 1
     sample for 0.01 absorbance, determined from the analysis of a
     0.75 µg/sample, using 5 cm optical path length cells.

3. Interferences

3.1 Any compound which has an absorbance maximum in the region of the
     ethylene thiourea complex will interfere by causing a higher
     absorbance reading.

4. Precision and Accuracy

4.1 The analytical precision has been determined from 21 spiked PVC
     filters ranging from 15-150 µg/sample. The analytical relative
     standard deviation (RSD) is 3%.

4.2   The average percent recovery is 98.7% determined from 21 spiked

PVC filters in the 15-150 µg/sample range.

5.   Advantages and Disadvantages

5.1   The method is simple, relatively fast, has very good sensitivity

and precision, and requires inexpensive instrumentation.

5.2   The pentacyanoammineferrate reagent will complex with compounds

containing the thione (C=S) moiety; this method, therefore,

will be applicable to those compounds containing this moiety.

5.3   The complexing reagent is stable for at least two weeks when

refrigerated.

5.4   Stability studies of the ethylene thiourea complex revealed

a 1.5% degradation of the color in 3 hours.

6.   Apparatus

6.1   Air Sampling Equipment

6.1.1   Vinyl Metricel (VM-1) filters, 5 µm in pore size, 37 mm

in diameter (Gelman Corp) or any equivalent filter.

6.1.2   Plastic three-piece 37 mm filter holders (cassettes)

(Millipore Corp.) or eqivalent.

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6.1.3 A personal air sampling pump capable of operating for

6 hours at 2.0 liters/min.

6.2 Visible Spectrophotometer.

6.3 Matched glass cells, 5 cm optical path length.

6.4 Analytical balance, capable of weighing to the nearest 0.1 mg.

6.5 Mortar and pestle.

6.6 Water bath, thermostatically controlled to ± 1 C.

6.7 Glass vials, 25 ml capacity, with teflon-coated screw caps.

6.8 Beakers, assortment of 50-250 ml.

6.9 Adjustable pipettes (5-50 μl, 50-250 μl, 0.5-5 ml) with disposable

tips.

6.10 Volumetric pipettes, assortment of 1-25 ml capacity.

6.11 Volumetric flasks, 25, 100, 200, and 250 ml capacity.

6.12 Miscellaneous: tweezers, microspatula, rubber suction bulb,

wood applicator stick, weighing paper, filter paper, and

filter funnel with stand.

7. Reagents

All reagents used should be ACS reagent grade or better.

7.1   Bromine liquid, Br2. (Caution: corrosive liquid, causes severe
      burns, vapors extremely irritating and toxic. Wear gloves and
      handle only in a hood).

7.2   Sodium nitroferricyanide (sodium nitroprusside), $Na_2Fe(CN)_5NO \cdot 2H_2O$.

7.3   Hydroxylamine hydrochloride, $NH_2OH \cdot HCl$

7.4   Sodium bicarbonate, $NaHCO_3$

7.5   Hexane

7.6   Methanol

7.7   Distilled or deionized water

7.8   Complexing Reagent

      7.8.1 Weigh 0.500 g sodium nitroferricyanide in a 50 ml beaker.
            Add 10 ml distilled water to the beaker and swirl to
            dissolve.

7.8.2 Weigh 0.500 g hydroxylamine hydrochloride and 1.000 g

sodium bicarbonate. Transfer both materials to a mortar

and carefully grind them together.

7.8.3 The next two steps must be performed in a hood. Add

the ground mixture to the sodium nitroferricyanide

solution. A bubbling reaction occurs for a few minutes.

When the reaction is complete, add 0.10 ml bromine (see

"caution" on Section 7.1). When effervescence stops, add

approximately 10 ml distilled water and filter the solution.

Rinse the beaker with 4 ml distilled water and filter.

Transfer filtrate to a 25 ml volumetric flask and dilute

with distilled water to the mark. This reagent should

be kept refrigerated.

7.8.4 Dilute complexing reagent. Mix one part of complexing

reagent with two parts water. Prepare this solution daily

when needed.

7.9   Ethylene thiourea (2-imidazolidinethione). Usually this

material is less than 99% pure and needs to be purified by

recrystallization.

7.9.1 Recrystallization. In a 250 ml Erlenmeyer flask, weigh

3-5 g ethylene thiourea. Add 100 ml 1:1 methanol-water

to dissolve the material. In a hood, heat the mixture to

boiling. Cool the flask at room temperature for five minutes. Add 5 ml hexane and shake the flask for 30 seconds. Cover the flask with a watch glass and leave undisturbed at room temperature for one hour. Filter the purified crystals, washing with 100 ml methanol-water mixture. Let the crystals air dry in the hood.

7.9.2 <u>Caution</u>: Ethylene thiourea has been found to be a teratogen and carcinogen in experimental animals. Extra care must be taken to avoid inhalation or skin contact with this material. Keep all material in a labeled bottle clearly identified as "potential human carcinogen," and place in a resealable thick-walled plastic bag, in locked storage.

7.10 Ethylene thiourea 1000 μg/ml stock solution.

7.10.1 Weigh 0.250 g of recrystallized ethylene thiourea in a beaker. Add distilled water to dissolve the material. Transfer the solution into a 250 ml volumetric flask and dilute with distilled water to the mark.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash all glassware with detergent solution, rinse with tap water, distilled water, and dry in an oven.

8.2 Calibration of sampling pump. The personal sampling pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within $\pm 5\%$ may be used for the calibration.

8.3 Collection and Shipping of Samples

8.3.1 Assemble the filters in the filter holder so that the air being sampled passes first through the PVC filter and then through the filter support. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing.

8.3.2 Sample at least 100 liters of air. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material may plug the filter, the flow rate should be checked at least once every hour. On completion of sampling, reinsert the small plugs into the inlet and outlet of the filter holder. Record the temperature and pressure of the air being sampled.

8.3.3 With each group of samples prepare two blanks consisting of a filter holder with a representative filter that has been handled in the same manner as the sample filters, except that no air is drawn through them. The samples and the blank should be shipped promptly in a damage-proof container that allows no filter holder movement. Samples should be refrigerated as soon as possible.

8.4 Analysis of Samples

8.4.1 Extraction

1. Remove the top portion of the filter holder. Hold the bottom portion containing the filter and filter support over a piece of weighing paper to catch any particulate material that may fall out. Remove the small plug from the bottom portion of the filter holder and insert the applicator stick through the hole. Gently raise the filter support and the filter and grasp the unexposed edge with tweezers. Very carefully pick up the filter, insert it in a glass vial, and push it gently to the bottom with the applicator stick. Add to the vial any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipet 7.0 ml of distilled water into the vial.

   This amount should completely cover the filter.

   Screw the cap on the vial.

3. Place vials in a 60 C water bath (thermostatically

   controlled) for 45 minutes. The water bath level

   must be above the water level of the vial. Shake

   each vial every 5 minutes. The use of ultrasonic

   bath is not recommended because it breaks up the

   PVC filter.

4. Lift the filter with tweezers so it is above the

   water level in the vial and wash the filter eight

   times with 1 ml aliquots of water using a 1 ml

   pipette. Position the filter over the center of

   the vial so the rinsings can be collected in the

   vial, then discard the filter.

8.4.2 Complexation

This step should be performed at the same time for both

standard and field samples so that the color formation

will start at the same time. This reduces color

degradation discrepancies.

1. Pipet a 1.5 ml aliquot of the dilute complexing

    reagent into each vial.

2. Allow the vials to stand for at least 30 minutes
before analysis to insure full color development.
Shake the vials every 10 minutes.

8.4.3 Analysis

1. Transfer the solution to a clean 5-cm optical path
length glass cell. Wipe off with a lens paper any
droplets left on the cell windows.

2. Place the cell in the sample compartment and
measure the absorbance at 590 nm. The reference
sample contains 15 ml distilled water and 1.5 ml
dilute complexing reagent in a 5-cm glass cell.
Record the absorbance for each sample.

8.5 Determination of Extraction Efficiency

8.5.1 The extraction efficiency of ethylene thiourea may vary
from laboratory to laboratory. The average percent
recovery determined from 21 spiked filter samples was
98.7% with a 3% relative standard deviation in the
15-150 µg/sample range.

8.5.2 Procedure

1. On a plastic test tube rack place eight PVC
   filters. Using an adjustable pipette, add to
   the center of each filter 0, 15, 30, 45, 60, 90,
   120, and 150 microliters of the 1000 µg/ml ethylene
   thiourea stock solution. This corresponds to
   concentrations of 0, 15, 30, 45, 60, 90, 120, and 150
   micrograms per filter. Let filters air-dry overnight
   at room temperature. Place each spiked filter in a
   vial, and mark its concentration. Follow sections
   8.4.1 parts 2 through 8.4.3 for preparation and
   analyses of the spiked samples.

2. The absorbance of each sample is converted to micro-
   grams from the calibration curve (Section 10.1).
   Percent recovery is determined as follows:

$$\text{Percent recovery} = \frac{\underline{\text{micrograms recovered}}}{\text{micrograms added}} \times 100$$

9. Calibration and Standardization

9.1 Ethylene thiourea 15 µg/ml standard solution.

9.1.1 Pipet 3 ml aliquot of the 1000 µg/ml stock solution
      into a 200 ml volumetric flask. Dilute with distilled
      water to the mark.

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9.2 Preparation of Standard Samples

    9.2.1 Prepare the standards by following Table I. Pipet

          aliquots of the 15 μg/ml standard solution and

          distilled water in a marked vial.

TABLE I

Volume 15 µg/ml std	Volume distilled water	Concentration
ml	ml	µg/16.5 ml sample solution
0.00	15.00	0
1.00	14.00	15
2.00	13.00	30
3.00	12.00	45
4.00	11.00	60
6.00	9.00	90
8.00	7.00	120
10.00	5.00	150

Standards contain 1.5 ml of dilute complexing reagent. Total volume is 16.5 ml/vial. Follow section 8.4.2 and 8.4.3 to complex and analyze the standards.

9.2.2 Standards must be prepared and analyzed in conjunction with field samples. Analyses of standards should be performed before and after field samples are analyzed.

9.2.3 Prepare a calibration curve by plotting absorbance vs concentration of standards in µg/16.5 ml sample.

10. Calculations

10.1 The concentration of ethylene thiourea in field samples can be determined graphically from the plot of absorbance vs concentration.

The concentration can also be determined using the following expression obtained from least squares analysis.

$$\text{Concentration in } \mu g/\text{sample} = \frac{\text{Absorbance} - y \text{ intercept}}{\text{slope}}$$

10.2 The concentration of ethylene thiourea in air may be expressed in mg/m^3.

$$mg/m^3 = \frac{\text{weight of field sample (in } \mu g)}{\text{volume of air sampled (in } l)}$$

10.3 For personal pumps with rotameters only, the following correction for air volumes sampled should be made:

$$\text{Corrected Volume} = f \times t \sqrt{\frac{P_1}{P_2} \times \frac{T_2}{T_1}}$$

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where:

f = sample flow rate

t = sampling time

P_1 = pressure during calibration of sampling pump (mm Hg)

P_2 = pressure of air sampled (mm Hg)

T_1 = temperature during calibration of sampling pump (K)

T_2 = temperature of air sampled (K)

11. References

11.1 Grote WI: A New Color Reaction for Soluble Organic
      Sulfur Compounds. J Biol Chem 93:25, 1931

11.2 Danowski TS: Measurement of Thiourea in Ultrafiltrate
      of Serum. J Biol Chem 152:201, 1944

11.3 Association of Official Agricultural Chemists, 31, 100, 1948

APPENDIX A

SAMPLING AND ANALYTICAL METHODS

There are a number of analytical techniques available in the literature which may be used to detect ETU in air, solutions, and solids. This multiplicity of methods has been largely due to the need of many investigators to detect minute amounts of ETU as contaminants or metabolic products of the ethylenebisdithiocarbamate fungicides.

The two main categories of detection have involved gas liquid chromatography (GLC), or wet chemistry (WC) methods, with GLC methods generally having greater sensitivity, specificity, and accuracy. However, most are best adapted for research rather than routine analysis.

The detectors utilized thus far in GLC have included electron capture (ECD), thermionic (TID), and flame photometric (FPD) detectors. While smaller sized samples may be used with the ECD, derivatization of the sample must usually be performed before detection. This step can be eliminated in the FPD method, but other FPD methods reported utilize derivatization which, of course, increases specificity. While selective GLC methods are more often used in research on ETU where confirmation of the identity of ETU is essential, it may also be used in routine analyses of air and solution samples as well. Solid samples nearly always require additional procedures.

In nearly all cases, the stepwise procedure for GLC analyses involves: (1) extraction, (2) cleanup, (3) derivatization, and (4) determination. Extraction is usually performed with methanol or ethanol but mixtures using acetone, chloroform, trichloroacetic acid and sodium ascorbate have also been used. Cleanup involves eliminating interfering substances. This is especially necessary in biological samples containing ETU. Cleanup is most efficiently done by separation of a derivative of ETU from an aqueous sample solution. Column chromatography or solvent partitioning may also be used (Onley and Yip, 1971).

In derivatization, the thio group is used for alkylation. This allows for the primary distinction among classes of chemicals similar to ETU. Various derivatives have been championed, each with various advantages. The final determination of the derivative is established by the partition coefficient along selected distinctive column packings.

Additional techniques for ETU analysis include: (1) thin layer chromatography (Vonk, 1970, Blazquez, 1973, Olney and Yip, 1971, and Engst, 1974) --this method generally has a lower detection limit of 20 ppb--;(2) polography (Engst, 1974) --here the nitroso derivative is determined after alumina column cleanup--; (3) Radioactive derivatization (Nash, unpublished); (4) Spectographic (NIOSH standard method, Palassi, 1977). This simple, as yet unpublished spectophotometric method for the determination of ETU is especially suited to measurement of air samples. It has the combined advantages of direct reading, good sensitivity, and adequate specificity (all thio

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groups). It is relatively fast and does not require expensive instrumentation. The NIOSH-verified method of analysis of ETU is presented below:

ETHYLENE THIOUREA

(2 Imidazolinethione)

Measurements Support Branch

Analytical Method

Analyte: Ethylene thiourea

Matrix:	Air	Range:	0.03-1.5 mg/m3
			for 100 1 sample
Procedure:	Filter collection, extraction		
	complexation, spectrophoto-	Precision:	3% (Analytical)
	metry		

1. Synopsis

Ethylene thiourea is collected from air on a PVC membrane filter. The filter is extracted with distilled water. Pentacyanoamine-ferrate reagent is added to the extract to form a colored coordination

complex. The absorbance of the solution is measured spectrophoto-metrically at 590 nm, and the concentration of ethylene thiourea is determined from a calibration curve.

2. Working Range, Sensitivity, and Detection Limit

2.1 The working range for this method is 0.03-1.5 mg/m3 in 100 1 sample. The entire linear working range has not been determined.

2.2 The sensitivity is 0.006 absorbance units/g, as determined from the slope of several calibration curves.

2.3 The detection limit is 0.75 g/sample or 0.0075 mg/m3 in 100 1 sample for 0.01 absorbance, determined from the analysis of a 0.75 g/sample, using 5 cm optical path length cells.

3. Interferences

3.1 Any compound which has an absorbance maximum in the region of the ethylene thiourea complex will interfere by causing a higher absorbance reading.

4. Precision and Accuracy

4.1 The analytical precision has been determined from 21 spiked PVC filters ranging from 15-150 g/sample. The analytical relative standard deviation (RSD) is 3%.

4.2 The average percent recovery is 98.7% determined from 21 spiked
PVC filters in the 15-150 g/sample range.

5. Advantages and Disadvantages

5.1 The method is simple, relatively fast, has very good sensitivity
and precision, and requires inexpensive instrumentation.

5.2 The pentacyanoammineferrate reagent will complex with compounds
containing the thione (C=S) moiety; this method, therefore,
will be applicable to those compounds containing this moiety.

5.3 The complexing reagent is stable for at least two weeks when
refrigerated.

5.4 Stability studies of the ethylene thiourea complex revealed
a 1.5% degradation of the color in 3 hours.

6. Apparatus

6.1 Air Sampling Equipment

6.1.1 Vinyl Metricel (VM-1) filters, 5 um in pore size, 37 mm
in diameter (Gelman Corp) or any equivalent filter.

6.1.2 Plastic three-piece 37 mm filter holders (cassettes)
(Millipore Corp.) or equivalent.

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6.1.3 A personal air sampling pump capable of operating for
6 hours at 2.0 liters/min.

6.2 Visible Spectrophotometer.

6.3 Matched glass cells, 5 cm optical path length.

6.4 - (deleted)

6.5 Mortar and pestle.

6.6 Water bath, thermostatically controlled to ±10C.

6.7 Glass vials, 25 ml capacity, with teflon-coated screw caps.

6.8 Beakers, assortment of 50-250 ml.

6.9 Adjustable pipettes (5-50 ml, 50-250 ml, 0.5-5 ml) with disposable
tips.

6.10 Volumetric pipettes, assortment of 1-25 ml capacity.

6.11 Volumetric flasks, 25, 100, 200, and 250 ml capacity.

6.12 Miscellaneous: tweezers, microspatula, rubber suction bulb,
wood applicator stick, weighing paper, filter paper, and
filter funnel with stand.

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7. Reagents

All reagents used should be ACS reagent grade or better.

7.1 Bromine liquid, Br2. (<u>Caution</u>: corrosive liquid, causes severe burns, vapors extremely irritating and toxic. Wear gloves and handle only in a hood).

7.2 Sodium nitroferricyanide (sodium nitroprusside), Na2Fe(CN)5NO.2H2O.

7.3 Hydroxylamine hydrochloride, NH2OH.HCl

7.4 Sodium bicarbonate, NaHCO3

7.5 Hexane

7.6 Methanol

7.7 Distilled or deionized water

7.8 Complexing Reagent

7.8.1 Weigh 0.500 g sodium nitroferricyanide in a 50 ml beaker. Add 10 ml distilled water to the beaker and swirl to dissolve.

7.8.2 Weigh 0.500 g hydroxylamine hydrochloride and 1.000 g

sodium bicarbonate. Transfer both materials to a mortar

and carefully grind them together.

7.8.3 The next two steps must be performed in a hood. Add

the ground mixture to the sodium nitroferricyanide

solution. A bubbling reaction occurs for a few minutes.

When the reaction is complete, add 0.10 ml bromine (see

"caution" on Section 7.1). When effervescence stops, add

approximately 10 ml distilled water and filter the solution.

Rinse the beaker with 4 ml distilled water and filter.

Transfer filtrate to a 25 ml volumetric flask and dilute

with distilled water to the mark. This reagent should

be kept refrigerated.

7.8.4 Dilute complexing reagent. Mix one part of complexing

reagent with two parts water. Prepare this solution daily

when needed.

7.9 Ethylene thiourea (2-imidazolidinethione). Usually this

material is less than 99% pure and needs to be purified by

recrystallization.

7.9.1 Recrystallization. In a 250 ml Erlenmeyer flask, weigh

3-5 g ethylene thiourea. Add 100 ml 1:1 methanol-water

to dissolve the material. In a hood, heat the mixture to

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boiling. Cool the flask at room temperature for five minutes.
Add 5 ml hexane and shake the flask for 30 seconds. Cover
the flask with a watch glass and leave undisturbed at room
temperature for one hour. Filter the purified crystals,
washing with 100 ml methanol-water mixture. Let the
crystals air dry in the hood.

7.9.2 Caution: Ethylene thiourea has been found to be a
teratogen and carcinogen in experimental animals. Extra
care must be taken to avoid inhalation or skin contact
with this material. Keep all material in a labeled bottle
clearly identified as "potential human carcinogen," and
place in a resealable thick-walled plastic bag, in locked
storage.

7.10 Ethylene thiourea 1000 g/ml stock solution.

7.10.1 Weigh 0.250 g of recrystallized ethylene thiourea in a
beaker. Add distilled water to dissolve the material.
Transfer the solution into a 250 ml volumetric flask
and dilute with distilled water to the mark.

8. Procedure

8.1 Cleaning of Equipment

8.1.1 Wash all glassware with detergent solution, rinse with tap water, distilled water, and dry in an oven.

8.2 Calibration of sampling pump. The personal sampling pump should be calibrated with a representative filter assembly in the line. A wet or dry test meter or a glass rotameter capable of measuring the flow rate to within $\pm 5\%$ may be used for the calibration.

8.3 Collection and Shipping of Samples

8.3.1 Assemble the filters in the filter holder so that the air being sampled passes first through the PVC filter and then through the filter support. Remove the small plugs from the filter holder and connect the filter holder to the sampling pump by means of an adapter and a length of tubing.

8.3.2 Sample at least 100 liters of air. The optimum sample volume will depend on the type of workroom environment being sampled. Since high concentrations of particulate material may plug the filter, the flow rate should be

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checked at least once every hour. On completion of
sampling, reinsert the small plugs into the inlet and
outlet of the filter holder. Record the temperature
and pressure of the air being sampled.

8.3.3 With each group of samples prepare two blanks consisting
of a filter holder with a representative filter that has
been handled in the same manner as the sample filters,
except that no air is drawn through them. The samples
and the blank should be shipped promptly in a damage-
proof container that allows no filter holder movement.
Samples should be refrigerated as soon as possible.

8.4 Analysis of Samples

8.4.1 Extraction

1. Remove the top portion of the filter holder. Hold
the bottom portion containing the filter and filter
support over a piece of weighing paper to catch any
particulate material that may fall out. Remove the
small plug from the bottom portion of the filter
holder and insert the applicator stick through the
hole. Gently raise the filter support and the filter
and grasp the unexposed edge with tweezers. Very
carefully pick up the filter, insert it in a glass

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vial, and push it gently to the bottom with the applicator stick. Add to the vial any particulate material remaining in the filter holder or collected on the weighing paper.

2. Pipet 7.0 ml of distilled water into the vial. This amount should completely cover the filter. Screw the cap on the vial.

3. Place vials in a 60C water bath (thermostatically controlled) for 45 minutes. The water bath level must be above the water level of the vial. Shake each vial every 5 minutes. The use of ultrasonic bath is not recommended because it breaks up the PVC filter.

4. Lift the filter with tweezers so it is above the water level in the vial and wash the filter eight times with 1 ml aliquots of water using a 1 ml pipette. Position the filter over the center of the vial so the rinsings can be collected in the vial, then discard the filter.

8.4.2 Complexation

This step should be performed at the same time for both standard and field samples so that the color formation

will start at the same time. This reduces color
degradation discrepancies.

1. Pipet a 1.5 ml aliquot of the <u>dilute</u> complexing
    reagent into each vial.

2. Allow the vials to stand for at least 30 minutes
    before analysis to insure full color development.
    Shake the vials every 10 minutes.

8.4.3 Analysis

1. Transfer the solution to a clean 5-cm optical path
    length glass cell. Wipe off with a lens paper any
    droplets left on the cell windows.

2. Place the cell in the sample compartment and
    measure the absorbance at 590 nm. The reference
    sample contains 15 ml distilled water and 1.5 ml
    dilute complexing reagent in a 5-cm glass cell.
    Record the absorbance for each sample.

8.5 Determination of Extraction Efficiency

8.5.1 The extraction efficiency of ethylene thiourea may vary from laboratory to laboratory. The average percent recovery determined from 21 spiked filter samples was 98.7% with a 3% relative standard deviation in the 15-150 g/sample range.

8.5.2 Procedure

1. On a plastic test tube rack place eight PVC filters. Using an adjustable pipette, add to the center of each filter 0, 15, 30, 45, 60, 90, 120, and 150 microliters of the 1000 g/ml ethylene thiourea stock solution. This corresponds to concentrations of 0, 15, 30, 45, 60, 90, 120, and 150 micrograms per filter. Let filters air-dry overnight at room temperature. Place each spiked filter in a vial, and mark its concentration. Follow sections 8.4.1 parts 2 through 8.4.3 for preparation and analyses of the spiked samples.

2. The absorbance of each sample is converted to micrograms from the calibration curve (Section 10.1). Percent recovery is determined as follows:

$$\text{Percent recovery} = \frac{\text{micrograms recovered}}{\text{micrograms added}} \times 100$$

9. Calibration and Standardization

    9.1 Ethylene thiourea 15 g/ml standard solution.

        9.1.1 Pipet 3 ml aliquot of the 1000 g/ml stock solution
into a 200 ml volumetric flask. Dilute with distilled
water to the mark.

    9.2 Preparation of Standard Samples

        9.2.1 Prepare the standards by following Table I. Pipet
aliquots of the 15 g/ml standard solution and
distilled water in a marked vial.

TABLE I

Volume 15 g/ml std	Volume distilled water	Concentration
ml	ml	g/16.5 ml sample solution
0.00	15.00	0
1.00	14.00	15
2.00	13.00	30
3.00	12.00	45
4.00	11.00	60
6.00	9.00	90
8.00	7.00	120
10.00	5.00	150

Standards contain 1.5 ml of dilute complexing reagent. Total volume is 16.5 ml/vial. Follow section 8.4.2 and 8.4.3 to complex and analyze the standards.

9.2.2 Standards must be prepared and analyzed in conjunction with field samples. Analyses of standards should be performed before and after field samples are analyzed.

9.2.3 Prepare a calibration curve by plotting absorbance vs concentration of standards in g/16.5 ml sample.

10. Calculations

10.1 The concentration of ethylene thiourea in field samples can be determined graphically from the plot of absorbance vs concentration.

10.2 The concentration of ethylene thiourea in air may be expressed in mg/m3 .

10.3 For personal pumps with rotameters only, the following correction for air volumes sampled should be made:

$$\text{Corrected Volume} = f \times t \sqrt{\frac{P_1}{P_2} \times \frac{T_2}{T_1}}$$

where:

f = sample flow rate

t = sampling time

P1= pressure during calibration of sampling pump (mm Hg)

P2= pressure of air sampled (mm Hg)

T1= temperature during calibration of sampling pump (K)

T2= temperature of air sampled (K)

11. References

11.1 Grote WI: "A New Color Reaction for Soluble Organic

      Sulfur Compounds," J. Biol. Chem., 93, 25, 1931

11.2 Danowski TS: "Measurement of Thiourea in Ultrafiltrate

      of Serum," J. Biol. Chem., 152, 201, 1944

11.3 Association of Official Agricultural Chemists, 31, 100, 1948

APPENDIX B: SYNONYMS AND TRADE NAMES OF ETU

Chemical Abstracts Service Registry Number 96-45-7
NIOSH RTECS Number NI96250
4,5-Dihydroimidazole-2(3H)-thione
4,5-Dihydro-2-mercaptoimidazole
N,N'-(1,2-Ethanediyl)thiourea
Ethylene thiourea
1, 3-ethylene-2-thiourea
N,N'-Ethylenethiourea
ETU
Imidazolidinethione
Imidazoline-2-thiol
Imidazoline-2(3H)-thione
2-imidazolidenethione
2-imidazolidene-2-thiol
Jor 4022
Mercaptoimidazoline
2-Mercaptoimidazoline
2-Mercapto-2-imidazoline
N, N'-ethylene-thiourea
NA-22
NA-22D
NA-22F
National Bureau of Standards Standard Reference Material 392
Pennac CRA
Robac 2.2
Rhodanin S-62
Sodium-22 neoprene accelerator
Tetrahydro-2H-imidazole-2-thione
Thiate-N
2-thiol-dihydroglyoxaline
Thiourea, N, N 1 (1, 2-ethandiyl)
2H-Imidazole -2-thione, tetrahydro
4,5-Dihydro-2-mercaptoimidazole
Vulkacit NPV/C
Warecure C

APPENDIX C

NIOSH ESTIMATE OF TYPES OF WORKERS EXPOSED TO ETU (Imidazolidinethione, 2-)
IN THE MANUFACTURE OF FABRICATED RUBBER PRODUCTS

National Occupational Hazard Survey (Projected) as of 3/7/78

Classification of Worker	No. of Exposures	% of Total
Industrial Engineers	30	0.2
Chemists	420	2.5
Personnel and Labor Relations Workers	60	0.4
Engineering and Science Technicians, N.E.C.	120	0.7
Research Workers, Not Specified	30	0.2
Managers and Administrators, N.E.C.	390	2.3
Shipping and Receiving Clerks	60	0.4
Weighers	246	1.4
Foremen, N.E.C.	600	3.6
Machinists	30	0.2
Heavy Equipment Mechanics, Incl. Diesel	246	1.4
Checkers, Examiners, and Inspectors; Manufacturing	930	5.6
Cutting Operatives, N.E.C.	330	2.0
Mixing Operatives	2,520	15.2
Packers and Wrappers, Except Meat and Produce	66	0.4
Painters, Manufactured Articles	66	0.4
Machine Operatives, Miscellaneous Specified	8,460	50.9
Miscellaneous Operatives	1,446	8.7
Not Specified Operatives	270	1.6
Freight and Material Handlers	150	0.9
Vehicle Washers and Equipment Cleaners	60	0.4
Miscellaneous Laborers	60	0.4
Not Specified Laborers	60	0.2
	16,620	100%

Table 1
Mean Body Weights (g) ± SE of Rats Fed ETU in the Diet
(Graham et al, 1975)

Dietary level, ppm	No. of rats	2 months of diet	No. of rats	6 months of diet	No. of rats	12 months of diet
			Males			
0	68	419 + 5	56	617 + 10	45	703 + 13
5	68	421 + 4	58	613 + 8	48	711 + 11
25	68	411 + 4	57	583 + 8a	47	670 + 12
125	68	421 + 5	58	601 + 9	46	692 + 12
250	67	403 + 4b	58	579 + 8b	44	677 + 12
500	68	327 + 4a	58	509 + 7a	47	604 + 15a
			Females			
0	68	270 + 3	58	371 + 6	47	466 + 11
5	68	267 + 3	58	363 + 7	48	464 + 11
25	68	259 + 3b	58	357 + 5	46	442 + 10
125	68	254 + 3a	58	342 + 5a	48	429 + 11c
250	68	245 + 3a	57	331 + 5a	46	408 + 9a
500	68	231 + 3a	58	326 + 5a	46	406 + 8a

a Significantly different from control value, p = 0.001.
b Significantly different from control value, p = 0.01.
c Significantly different from control value, p = 0.05.

Table 2

Mean Organ-to-Body Weight (g/kg) Ratios ± SE of Charles River Rats Fed
ETU for 2 Months, 6 months, and 12 months. (Ten Rats per Group)
(Graham et al, 1975)

Dietary level, ppm	2 Months Thyroid(a)	6 Months Thyroid(a)	12 Months Thyroid(a)
Males			
0	44.7 + 2.3	51.5 + 3.3	44.3 + 2.0
5	45.4 + 3.5	45.1 + 2.4	43.9 + 3.3
25	40.1 + 1.9	44.3 + 2.3	48.8 + 2.9
125	55.9 + 4.9	61.9 + 4.1	52.9 + 3.5b
250	93.6 + 5.9d	79.9 + 6.1c	87.7 + 13.2c
500	102.9 + 8.3d	139.5 + 14.9b	779.0 + 231.4b
Females			
0	60.0 + 2.9	59.1 + 3.1	57.3 + 3.5
5	66.8 + 3.5	65.9 + 4.4	56.4 + 3.3
25	66.2 + 3.3	59.8 + 3.8	56.8 + 3.3
125	78.4 + 3.3d	72.0 + 4.6d	68.6 + 4.1b
250	105.5 + 5.3d	93.5 + 4.1b	97.7 + 8.0e
500	171.3 + 15.51	174.6 + 21.5b	271.5 + 85.7b

a Thyroid ratios are expressed as mg/kg.
b Significantly different from control, p = 0.05.
c Significantly different from control, p = 0.01.
d Significantly different from control, p = 0.001.

Table 3

THYROID HORMONE LEVELS - AFTER 30 DAYS ON STUDY (Freudenthal et al, 1977)

ETU (ppm)	Sex	125-I (percent uptake)	TBG (percent T-3 bound)	T-3 (ng percent)	T-4 (ug percent)	TSH (uIU per ml)
625	M	1.2 ± 0.4*	60.3 ± 2.6*	57.3 ± 3.7*	0.9 ± 0.6*	14.3 ± 0.9*
625	F	2.1 ± 1.4*	60.6 ± 1.8	58.4 ± 9.9*	1.1 ± 1.0*	14.6 ± 1.9*
125	M	3.6 ± 0.8	60.7 ± 1.3*	71.1 ±11.8	2.6 ± 0.4*	23.3 ± 5.9
125	F	4.0 ± 1.7	61.5 ± 1.3*	104.4 ±16.3*	2.1 ± 0.5*	18.3 ± 4.0*
25	M	2.9 ± 0.6	65.7 ± 2.3	67.1 ±15.9	5.6 ± 1.1	7.3 ± 1.5
25	F	3.2 ± 1.3	63.5 ± 2.0	86.3 ±14.8	3.8 ± 0.8	5.1 ± 1.3
5	M	3.6 ± 0.6	69.3 ± 6.3	79.0 ± 8.1	4.7 ± 0.4	6.7 ± 1.4
5	F	3.8 ± 1.0	68.9 ± 1.3	88.1 ±12.8	2.9 ± 0.9	4.9 ± 1.4
1	M	3.7 ± 0.7	64.5 ± 1.2	82.1 ±13.0	5.1 ± 1.0	6.4 ± 0.8
1	F	3.0 ± 0.5	63.4 ± 1.3	90.9 ±11.3	3.5 ± 1.0	4.5 ± 0.9
0	M	3.6 ± 0.9	68.0 ± 5.6	76.0 ±11.8	5.0 ± 1.7	6.7 ± 2.5
0	F	3.5 ± 0.9	66.0 ± 5.2	83.2 ±16.2	3.8 ± 1.4	6.0 ± 4.1
PTU (propyl thiourea) (ppm)						
125	M	2.9 ± 1.1	67.8 ± 2.0	58.9 ± 6.1*	0.9 ± 0.2*	--
125	F	3.3 ± 0.7	69.5 ± 1.6	52.0 ± 8.0*	0.7 ± 0.1*	

*Significantly different (p = 0.05) from corresponding control. Student's t test was used to make comparison between the control and treated animals. All data reported as the mean, ± S.D.

Table 4
THYROID HORMONE LEVELS - AFTER 60 DAYS ON STUDY (Freudenthal et al, 1977)

ETU (ppm)	Sex	125-I (percent uptake)	TBG (percent T-3 bound)	T-3 (ng Percent)	T-4 (ug percent)	THS (uIU per ml)
625	M	1.9 + 1.0*	79.0 + 0.9	56.9 + 10.3*	0.2 + 0.1*	-
625	F	2.4 + 1.8*	71.8 + 1.4	56.8 + 6.9*	0.2 + 0.1*	-
125	M	3.6 + 1.4	66.3 + 1.3	79.8 + 28.1	2.8 + 0.5*	
125	F	3.3 + 1.0	66.3 + 2.1	78.5 + 28.6*	2.0 + 0.5*	-
25	M	3.2 + 0.7	76.9 + 1.6	86.4 + 7.6	2.8 + 0.5*	-
25	F	3.7 + 1.3	74.7 + 1.7	126.2 + 15.1	2.6 + 0.5*	-
5	M	3.5 + 0.8	66.4 + 1.2	85.4 + 12.7	4.9 + 0.5	-
5	F	4.0 + 0.8	64.0 + 1.8	118.5 + 14.3	2.9 + 0.9	-
1	M	2.7 + 0.6	70.4 + 1.2	80.3 + 12.0	4.9 + 0.7	-
1	F	3.2 + 0.7	67.1 + 1.3	93.3 + 13.5	2.8 + 0.8	-
0	M	4.3 + 0.9	73.6 + 4.9	77.3 + 8.5	4.8 + 0.7	5.8 + 0.4**
0	F	3.5 + 0.8	69.4 + 4.3	103.8 + 19.1	3.3 + 0.5	6.4 + 0.9**
PTU (ppm)						
125	M	3.9 + 1.6	61.7 + 2.6*	46.1 + 3.9*	1.2 + 0.2*	9.8 + 1.0*
125	F	5.4 + 1.7	62.2 + 2.1*	50.9 + 9.7*	0.8 + 6.1*	10.8 + 1.9*

* Significantly different (p 0.05) from corresponding control.
** TSH values to be used as control for PTU group.

Table 5

THYROID HORMONE LEVELS - AFTER 90 DAYS ON STUDY (Freudenthal et al, 1977)

ETU (ppm)	Sex	125-I (percent uptake)	TBG (percent T-3 bound)	T-3 (ng percent)	T-4 (ug percent)	TSH (uIU per ml)
625	M	2.5 + 0.8*	62.7 + 2.0*	27.9 + 13.3*	1.1 + 0.6*	-
625	F	3.7 + 1.8	62.7 + 0.9*	35.2 + 4.3*	1.1 + 0.6*	-
125	M	2.8 + 0.7	65.3 + 1.1	86.1 + 15.0	2.3 + 0.6*	-
125	F	3.9 + 1.1	64.3 + 1.6	105.5 + 16.0	1.6 + 0.3*	-
25	M	3.3 + 0.7	68.9 + 1.5	79.4 + 12.6	3.8 + 1.0	-
25	F	3.4 + 0.9	65.6 + 2.3	108.7 + 11.6	2.9 + 0.7	-
5	M	3.7 + 0.6	71.4 + 0.8	76.1 + 13.1	5.0 + 1.0	-
5	F	4.2 + 1.1	70.1 + 2.2	105.2 + 16.6	3.0 + 0.7	-
1	M	3.5 + 0.6	65.8 + 1.1	68.7 + 9.9	4.0 + 1.0	-
1	F	3.2 + 0.9	63.1 + 1.4	116.7 + 17.6	2.5 + 0.7	-
0	M	3.8 + 0.5	69.3 + 2.7	72.0 + 21.5	4.5 + 0.8	5.8 + 0.4**
0	F	4.1 + 1.0	65.2 + 2.9	106.8 + 25.0	3.3 + 0.8	6.4 + 0.9**
PTU (ppm)						
125	M	4.7 + 1.7	59.1 + 1.3*	73.2 + 9.9	0.6 + 0.2*	9.4 + 1.3*
125	F	5.6 + 2.5	60.4 + 1.7*	69.6 + 9.4*	0.4 + 0.2*	10.7 + 2.1*

* Significantly different (p = 0.05) from corresponding control.
**TSH values to be used as control for PTU group.

Table 6

Relative risk for development of tumors among mice treated with ETU when compared with negative controls
(Innes et al, 1969)

Compound	Strain*	Mice with hepatomas			Mice with pulmonary tumors			Mice with lymphomas			Total mice with tumors		
		M	F	Sum	M	F	Sum	M	F	Sum	M	F	Sum
ETU	X	48.79*	Inf.+>	91.53+	1.31	5.45	3.03	0	1.59	1.06	14.82+	Inf.+>	27.44+
	Y	Inf.+>	68.82+	127.36+	1.16	0	1.05	13.47	6.28&	8.16+	Inf.+>	27.96+	38.18+
	Sum	82.08+	158.90+	106.06+	1.22	3.49	1.87	4.52	3.83&	4.04&	24.04+	42.93+	32.75+

*Strain X-(C57BL/6 x C3H/Anf) Fl;   strain Y-(C57BL/6 x AKR)Fl.
+Increased tumor yield significant at 0.01 level.
>Inf.- relative risk calculated as infinite. This figure may result
  from the absence of tumors in the control group and is not necessarily significant.
&Increased tumor yield significant at 0.05 level.