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Thyroid Follicular Cell Carcinogenesis: Mechanistic and Science Policy Considerations

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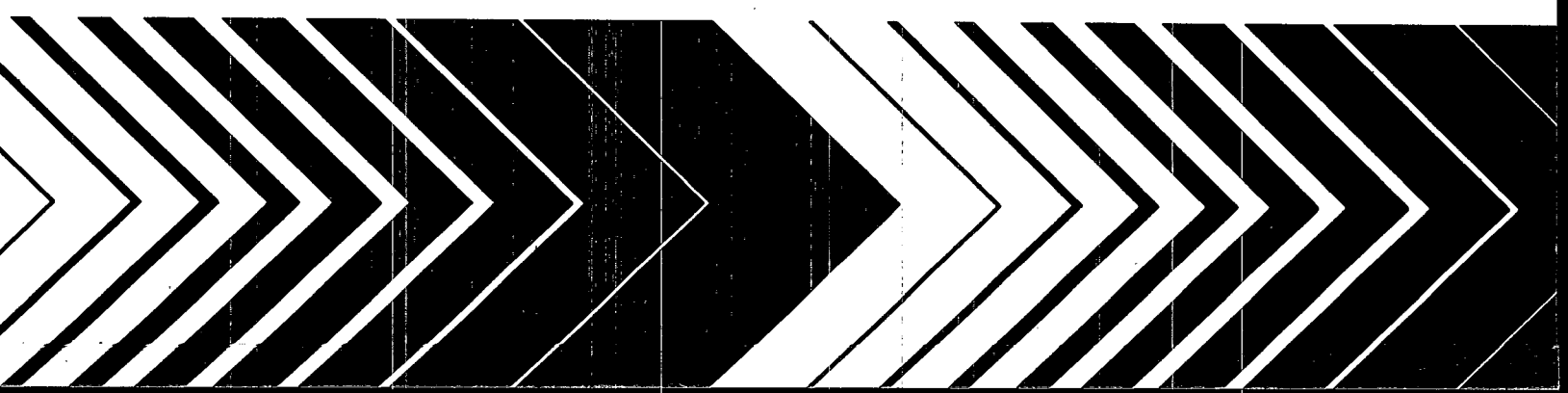

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THYROID FOLLICULAR CELL CARCINOGENESIS:

MECHANISTIC AND SCIENCE POLICY CONSIDERATIONS

Prepared for the
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PREFACE

The U.S. Environmental Protection Agency (EPA) Risk Assessment Forum was established to promote scientific consensus on risk assessment issues and to ensure that this consensus is incorporated into appropriate risk assessment guidance. To accomplish this, the Risk Assessment Forum assembles experts from throughout the EPA in a formal process to study and report on these issues from an Agency-wide perspective.

For major risk assessment activities, the Risk Assessment Forum may establish a Technical Panel to conduct scientific review and analysis. Members are chosen to assure that necessary technical expertise is available. Outside experts may be invited to participate as consultants or, if appropriate, as Technical Panel members.

The scientific analysis and policy recommendations in this report on thyroid neoplasia are based mainly on laboratory studies in which thyroid tumors in animals exposed to exogenous chemicals were associated with disruption in normal thyroid-pituitary function. The Forum analysis enlarges upon a 1986 Office of Pesticide Programs report on this issue and develops science policy recommendations for Agency-wide use.

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The Technical Panel acknowledges with appreciation the special contributions of Ms. Karlene Thomas and Ms. Pamela Bassford.

I. EXECUTIVE SUMMARY

A Technical Panel of the U.S. Environmental Protection Agency's (EPA) Risk Assessment Forum investigated potential mechanisms of action of agents that cause thyroid follicular tumors in animals and potentially in humans in an effort to develop a scientifically plausible approach for assessing risk due to exposure to these agents. Based on its review of relevant scientific information, the Technical Panel concluded that:

(1) thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary hormonal feedback under conditions of reduced circulating thyroid hormone and elevated thyroid stimulating hormone (TSH);

(2) the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and

(3) models that assume thresholds may be used to assess the risks of certain thyroid follicular cell tumors where there is evidence of thyroid-pituitary imbalance.

The policy set out in this report provides guidance on determining whether it is reasonable to presume that the observed thyroid follicular tumors are the result of thyroid-pituitary imbalance, and on selecting appropriate procedures to use in estimating the risks related to these tumors.

The scientific information reviewed by the Technical Panel provides sufficient evidence to support the conclusion that a threshold mechanism is likely to apply to the development of certain thyroid follicular tumors. In particular, several different types of experimental treatments in laboratory animals (e.g., iodide deficiency, subtotal thyroidectomy, chemical goitrogens) result in the formation

of thyroid tumors and to some extent pituitary tumors of the cells that secrete TSH, seemingly by the same mechanism. Tumors arise under conditions in which there is prolonged decrease in circulating thyroid hormone and increase in TSH. Under continued TSH stimulation, thyroid follicular cells undergo hypertrophy, hyperplasia and, eventually, neoplasia. It appears that TSH, probably in concert with other factors (e.g., somatomedins), acts as a stimulus for cell division, thus increasing the pool of cells at risk for neoplastic transformation. TSH may also play a role in the transformation process by yet undiscovered means.

Studies in humans reveal that they respond as do animals in regard to goitrogenic stimuli (e.g., iodide deficiency, thionamides); there is cellular hypertrophy and hyperplasia. Although thyroid enlargements and nodules may be risk factors for cancer development in humans, the case for neoplastic conversion under goitrogenic stimulation is less well established in humans than in animals. This suggests that humans may be less sensitive to the carcinogenic effects of long-term TSH stimulation than animals.

In its assessment of the relevant information, the Technical Panel focused on the following evidence: (1) a progression of events occurring under long-term exposure to an agent, including a disruption in thyroid-pituitary homeostasis involving reduction in thyroid hormone concentrations and increase in TSH levels, follicular cell hypertrophy and hyperplasia, benign follicular cell neoplasia and, possibly, malignant follicular cell neoplasia; (2) reversibility of certain steps in the progression when thyroid-pituitary homeostasis is reestablished; and (3) lack of consistent correlation of thyroid carcinogenicity with the genotoxic potential of chemical classes implicated in thyroid cancer. Based on this primary evidence, the Technical Panel developed a policy for risk assessment of agents that cause thyroid follicular cell tumors.

Briefly, the Technical Panel determined that threshold models may be applied in dose-response assessments for those chemical substances where only thyroid tumors (and relevant pituitary tumors) have been produced; the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis: and mechanisms other than thyroid-pituitary imbalance, e.g., genotoxicity, can be ruled out. Where there are tumors at other sites and/or genotoxicity is present, it is presumed that threshold models will not be used; however, case-by-case determinations are possible. Threshold models will not be used where there is no evidence of thyroid-pituitary imbalance.

Finally, the Technical Panel advised that in evaluating thyroid follicular cell neoplasms under this policy, the risk assessment depends on full use of the available information. In any given organism, a carcinogen may act through more than one mechanism at one or multiple anatomical sites. Accordingly, while use of this policy may be appropriate for assessing certain thyroid follicular cell tumors, use of other models may be necessary to evaluate risks at other tumor sites observed in the same study, which may result in different risk estimates. It is incumbent upon the risk assessor to consider all relevant risk estimates in making the final judgments on the potential human risk related to exposure to the chemical being evaluated.

II. INTRODUCTION

Responding to a request from the Office of Pesticides and Toxic Substances, the Risk Assessment Forum established a Technical Panel to study issues raised in an Office of Pesticide Programs (OPP) report on neoplastic changes in the thyroid gland. Thyroid follicular neoplasia [1/], the subject of this report, is a form that has been associated with low iodine diets, subtotal thyroidectomy, radioactive iodine, natural goitrogens such as rape seed and cabbage, chemotherapeutic agents such as sulfathiazole, pesticides such as amitrole, industrial chemicals like polychlorinated biphenyls, and contaminants like 2,3,7,8-tetrachlorodibenzo-p-dioxin. All of these agents either directly or indirectly interfere with the normal thyroid-pituitary feedback system.

In the OPP report, "Neoplasia Induced by Inhibition of Thyroid Gland Function (Guidance for Analysis and Evaluation)," Paynter et al., (1986) postulated that there is a causal relationship between thyroid-pituitary dysfunction and thyroid follicular neoplasia, and further that the mechanism underlying this relationship may be a threshold phenomenon. If this were the case, thyroid follicular carcinogenesis would not be expected to occur below a demonstrable threshold level of thyroid-pituitary dysfunction.

Simply stated, the OPP report described a possible mechanism for thyroid follicular neoplasia that involves interference with the normal physiological thyroid-pituitary hormonal feedback mechanism. It is postulated that certain

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1/ This report deals with mechanistic considerations surrounding the development of tumors of the parenchymal cells of the thyroid. In the experimental animal literature, such tumors are usually called follicular cell adenomas and carcinomas. The clinical literature usually divides human follicular cell tumors into different classes depending upon their histological features: follicular, papillary, and anaplastic. Neoplasms of the calcitonin-secreting parafollicular or C-cells (i.e., medullary tumors) are not considered in this report.

chemicals may result in decreased levels of thyroid hormone 2/ in the blood which result in increased release of thyroid stimulating hormone (TSH) by the anterior pituitary. This, in turn, leads to hypertrophy and hyperplasia of the thyroid without a corresponding increase in blood thyroid hormone levels; hyperplasia of the pituitary is also sometimes observed due to the reduced levels of circulating thyroid hormone. After prolonged stimulation of the thyroid-pituitary axis, thyroid (and to some extent, pituitary) hyperplasia may progress to neoplasia. Cessation of exposure prior to the induction of neoplasia results in a return toward the normal state. Because some degree of thyroid-pituitary dysfunction can be accommodated within the bounds of the normal feedback mechanism without induction of hyperplasia, a threshold for thyroid follicular cell carcinogenesis via hyperplasia appears to be indicated. Thus, for a chemical substance that decreases thyroid hormone levels, a dose below which it has any effect on thyroid pituitary hormone status may be conceived of as a threshold for the thyroid carcinogenic process.

Forum review of the issues raised in the OPP report was considered appropriate because of the potentially significant implications for carcinogenic risk assessment inherent in the OPP hypothesis. A risk assessment approach based on thyroid follicular neoplasia being a threshold phenomenon would be a significant departure from EPA's customary carcinogen risk assessment practice, which generally uses "nonthreshold" models for extrapolation from high- to low-dose exposures, based on the assumption that human carcinogenesis may develop as a result of exposure to carcinogens even at the very lowest levels. EPA's risk assessment guidelines recommend the linearized multistage model for

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2/ In this report, "thyroid hormone" is often used as a collective term to refer to the active thyroid hormones released from the thyroid gland into the circulation (thyroxine and 3,5,3'-triiodothyronine).

carcinogen risk assessment to place an upper bound on potential cancer risks, in the absence of relevant biological and statistical information to the contrary (U.S. EPA, 1986). However, the guidelines also stress that all of the available mechanistic, toxicological, metabolic, and pharmacokinetic information should be reviewed for each chemical in making judgments about the appropriateness, selection, and use of various extrapolation models.

The Technical Panel undertook the present analysis with three objectives: (1) to explore the role of thyroid-pituitary relationships in thyroid carcinogenesis; (2) to determine if threshold concepts might apply to the steps leading to thyroid cancer and (3) if warranted, to develop Agency-wide guidance on how threshold considerations may affect the estimation of risks from exposure to chemicals that produce thyroid tumors.

The Technical Panel has studied the OPP report, as well as an extensive number of additional studies and other information sources in order to assess whether the hypothesis set forth in the report is consistent with available information on human and animal thyroid neoplasia, thyroid-pituitary physiology and function, and the mechanisms of carcinogenesis. Upon review of such evidence, the Technical Panel agrees that under certain circumstances neoplasia in thyroid follicular cells involves interference of thyroid-pituitary feedback mechanisms and may involve threshold rather than nonthreshold processes. It is recognized that when there is evidence that the thyroid follicular tumors are related to an ordered linkage of steps from interference in thyroid-pituitary status leading to depressed thyroid hormone concentrations, elevated TSH levels, thyroid hypertrophy and hyperplasia, and neoplasia (adenoma and possibly carcinoma), then the threshold for an earlier step becomes a threshold for the entire chain of events. This Risk Assessment Forum report presents the findings of the Technical Panel.

The report has nine sections. Section I is an Executive Summary and this introduction constitutes Section II. Section III summarizes information on thyroid-pituitary physiology and biochemistry, and the hormonal feedback relationship between these glands. Section IV reviews the available information on the induction of thyroid follicular neoplasia, and sets forth a hypothetical mechanistic model based on current information on molecular and cellular processes relating to thyroid carcinogenesis. In Section V, exogenous factors affecting thyroid carcinogenesis are discussed, focusing primarily on information developed in experimental animals. Thyroid hyperplasia and neoplasia in humans are discussed in Section VI, and Section VII develops a science policy to guide the development of EPA risk assessments on this issue. Finally, Sections VIII and IX are the Appendices and References, respectively.

III. THYROID-PITUITARY PHYSIOLOGY AND BIOCHEMISTRY

In order to examine the possible role of pituitary, thyroid, and related hormones in thyroid carcinogenesis, it is important to first understand the physiology and biochemistry of the thyroid-pituitary hormonal system. Accordingly, this section summarizes the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated. References are mainly to recent reviews (see especially Paynter et al., 1986) rather than to the original scientific literature.

A. SYNTHESIS OF THYROID HORMONES

The thyroid hormones are synthesized in the thyroid gland and are stored as amino acid residues of thyroglobulin, a protein constituting most of the colloid in the thyroid follicles (Goodman and Van Middlesworth, 1980; Taurog, 1979; Haynes and Murad, 1985). Thyroglobulin is a complex glycoprotein made up of two identical subunits each with a molecular weight of 330,000 daltons.

The first stage in the synthesis of the thyroid hormones is the uptake of iodide from the blood by the thyroid gland (Figure 1). Uptake is active in nature (requires energy) and is effected by the so-called "iodide pump." Under normal conditions the thyroid may concentrate iodide up to about 50-fold its concentration in blood, and this ratio may be considerably higher when the thyroid is active. Iodide uptake may be blocked by several anions (e.g., thiocyanate and perchlorate) and, since iodide uptake involves concurrent uptake of potassium, it can be also blocked by cardiac glycosides that inhibit potassium accumulation.

The next step in the process is a concerted reaction in which iodide is oxidized to an active iodine species that in turn iodinates the tyrosyl residues of thyroglobulin. The reaction is effected by a heme-containing peroxidase in

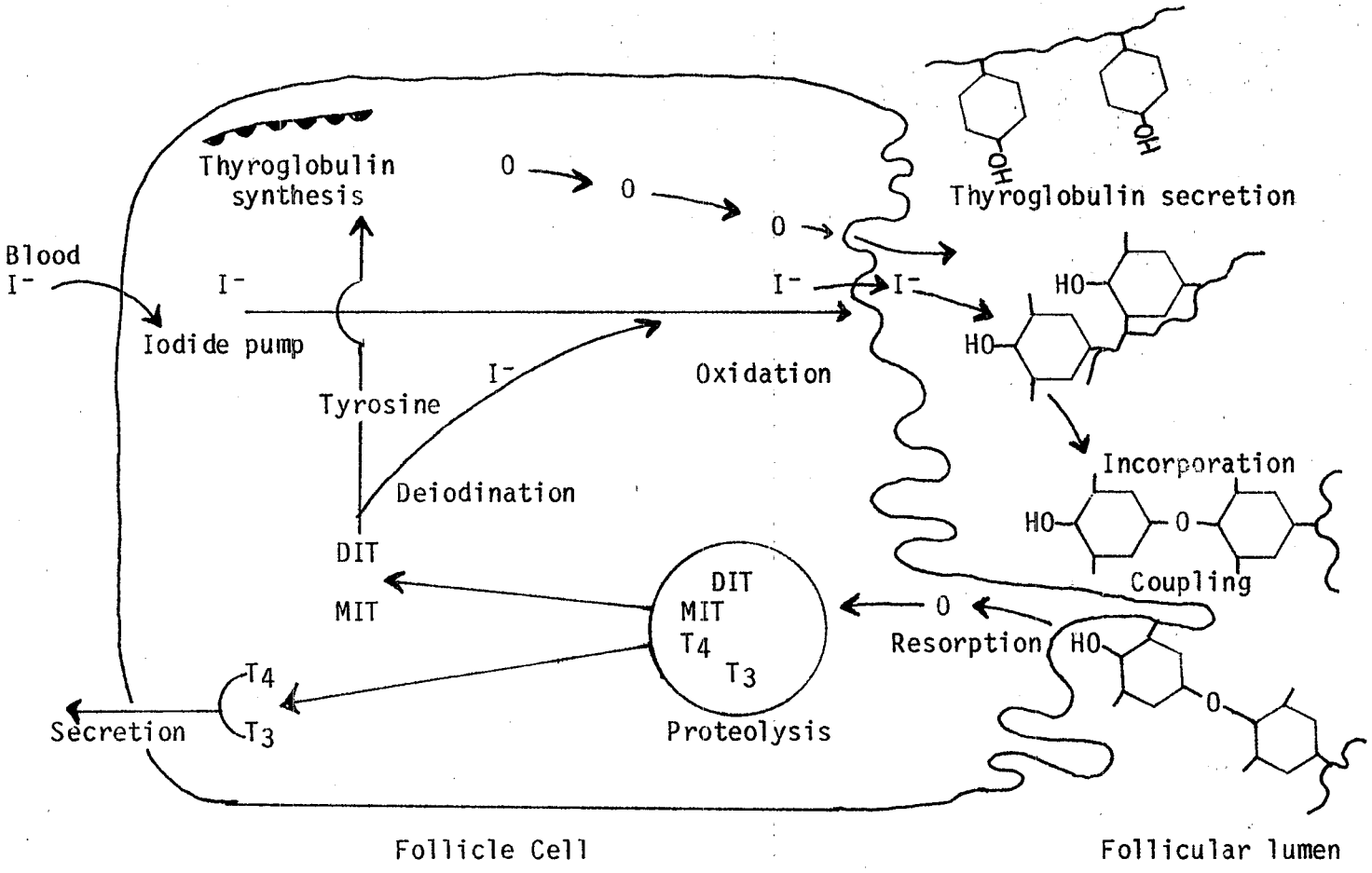


Figure 1. Schematic representation of thyroid hormone biosynthesis and secretion. Protein portion of thyroglobulin is synthesized in rough endoplasmic reticulum. It then travels to Golgi apparatus, where carbohydrate moieties are added, and proceeds to the apical surface in secretory vesicles, which fuse with the apical membrane and discharge their contents into the lumen. Iodide is pumped into the cell of a peroxidase. At the apical surface, it is oxidized through the action of a peroxidase. Iodine attaches to tyrosine residues in peptide linkage in thyroglobulin. Two iodinated tyrosyl groups couple in ether linkage to form thyroxine, which is still trapped in peptide linkage within thyroglobulin. The secretory process requires that thyroglobulin be engulfed by pseudopods thrown out into follicular lumen to resorb thyroglobulin into vesicles that fuse with lysosomes. Lysosomal protease breaks thyroglobulin down to amino acids, T_4, T_3, MIT, and DIT. T_4 and T_3 are released from the cell. DIT and MIT are deiodinated to free tyrosine and iodide, both of which are recycled back to iodinated thyroglobulin. (DIT = Diiodotyrosine; MIT = Monoiodotyrosine).

Source: Goodman and Van Middlesworth, 1980.

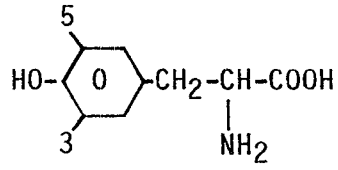
the presence of hydrogen peroxide. While diiodotyrosyl (DIT) residues constitute the major products, some monoiodotyrosyl (MIT) peptides are also produced (Figure 2). Additional reactions involving the coupling of two DIT residues or of one DIT with one MIT residue (each with the net loss of alanine) lead to peptides containing residues of the two major thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), respectively (Figure 1). It is thought that these reactions are catalyzed by the same peroxidase effecting the iodination reaction, and it seems that both peroxidase steps are blocked by certain compounds such as thiourea and some sulfonamides.

The release of T_4 and T_3 from thyroglobulin or smaller peptides is effected by endocytosis of colloid droplets into the follicular epithelial cells and subsequent action of lysosomal proteases. The free hormones are subsequently released into the circulation. It is not known whether thyroglobulin must be hydrolyzed completely to permit release of T_4 and T_3.

Although T_4 is by far the major thyroid hormone secreted by the thyroid (normally about 8 to 10 times the rate of T_3, although it varies as a function of the iodine intake), it is usually considered to be a prohormone. Thus, T_3 is about fourfold more potent than T_4, and about 33 percent of the T_4 secreted undergoes 5'-deiodination to T_3 in the peripheral tissues; another 40 percent undergoes deiodination of the inner ring to yield the inactive material reverse T_3 (Figure 2).

B. TRANSPORT OF THYROID HORMONES IN BLOOD

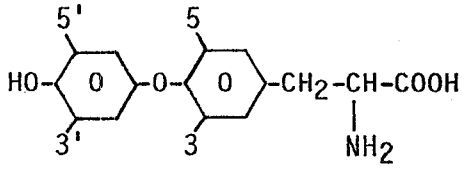
On entering the circulation, both T_4 and T_3 are transported in strong, but not covalent, association with plasma proteins (Figure 3). The major carrier-protein is thyroxine-binding globulin, a glycoprotein (M.W. 63,000) that forms a 1:1 complex with the thyroid hormones. Thyroxine-binding globulin has a very high affinity for T_4 (K_a about 10^{10} M) and a lower affinity for T_3. Thyroxine-



tyrosine

Monoiodotyrosine (MID) = 3-iodotyrosine

Diiodotyrosine (DID) = 3,5-diiodotyrosine



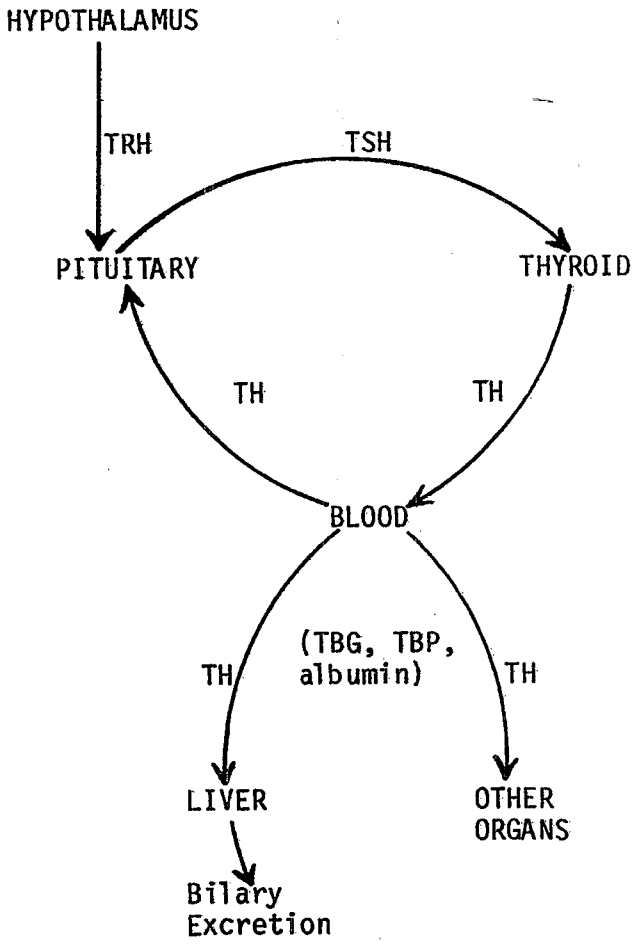
thyronine

Thyroxine (T_4) = 3,5,3',5'-tetraiodothyronine

Triiodothyronine (T_3) = 3,5,3'-triiodothyronine

Reversed triiodothyronine (rT_3) = 3,3',5'-triiodothyronine

Figure 2. Iodinated compounds of the thyroid gland.



TRH--thyrotropin releasing hormone

TSH--thyroid stimulating hormone

TH-- thyroid hormones

TBG--thyroxine binding globulin

TBP--thyroxine binding prealbumin

Figure 3. Hypothalamic-pituitary-thyroid-peripheral organ relationships.

binding prealbumin and albumin also transport thyroid hormones in the blood; the prealbumin has K_a values of about 10^7 M and 10^6 M for T_4 and T_3, respectively. Only about 0.03 percent of the T_4 in the circulation is free and available for cell membrane penetration and thus hormone action, metabolism, or excretion. The levels of free thyroid hormones in the circulation may be changed through competitive binding interactions of certain drugs and other foreign compounds (Haynes and Murad, 1985).

C. METABOLISM AND EXCRETION

As previously discussed, T_4, the major hormone secreted from the thyroid, is considered to be a prohormone and is converted to the more active T_3 by 5'-monodeiodination in a variety of peripheral tissues. T_4 is also metabolized to reverse T_3 which is hormonally inactive and has no known function, except perhaps as an inhibitor of the conversion of T_4 to T_3. Under normal conditions the half-life of T_4 is 6 to 7 days in humans.

Degradative metabolism of the thyroid hormones occurs primarily in the liver and involves conjugation with either glucuronic acid or sulfate through the phenolic hydroxyl group. The resulting conjugates are excreted in the bile into the intestine. A portion of the conjugated material is hydrolyzed in the intestine, and the free hormones thus released are reabsorbed into the blood (enterohepatic circulation). The remaining portion of the conjugated material (20% to 40% in humans) is excreted in the feces.

D. PHYSIOLOGIC ACTIONS OF THYROID HORMONES

While not of direct relevance to this discussion, the thyroid hormones play numerous and profound roles in regulating metabolism, growth, and development and in the maintenance of homeostasis. It is generally believed that these actions result from effects of the thyroid hormones on protein synthesis.

There is considerable evidence to suggest that many of the various biological effects of the thyroid hormones are initiated by the interaction of T_3 with specific nuclear receptors in target cells, presumably proteins (Oppenheimer, 1979). Recent evidence points to these receptors being the products of the c-erb-A oncogene (Weinberger et al., 1986; Sap et al., 1986). Such interactions can lead, directly or indirectly, to the formation of a diversity of mRNA sequences and ultimately to the synthesis of a host of different enzyme proteins. Qualitative and quantitative differences in the responses resulting from formation of T_3-receptor complexes may occur in different target tissues. Such differences may be controlled at a local cellular level and may be mediated through metabolic or hormonal factors.

E. REGULATION OF THYROID HORMONE SYNTHESIS/SECRETION

Homeostatic control of thyroid hormone synthesis and secretion in the thyroid gland is effected by a sensitive feedback mechanism that responds to changes in circulating levels of the thyroid hormones T_4 and T_3. The mechanism involves the hypothalamus and anterior pituitary of the brain (Figure 3) (Paynter, et al., 1986; Larsen, 1982; Houk, 1980)

Of central importance in the feedback mechanism is the thyroid stimulating hormone (TSH, thyrotropin), which is secreted by the anterior pituitary gland and causes the thyroid to initiate new thyroid hormone synthesis. Increases in iodide uptake, the iodination of thyroglobulin, and endocytosis and proteolysis of colloid are all observed in response to TSH stimulation. The effects of TSH on the thyroid appear to be the consequence of binding to cell-surface receptors and activation of adenyl cyclase and protein kinase with subsequent phosphorylation of cellular proteins. Cyclic adenosine monophosphate (cAMP) can itself mimic most of the actions of TSH on thyroid cells (Van Sande et al., 1983; Roger and Dumont, 1984). Further details of the molecular biology of TSH

action on the thyroid are discussed elsewhere in this document (Section IV.C.).

The rate of release of TSH from the pituitary is delicately controlled by the amount of thyrotropin-releasing hormone (TRH) secreted by the hypothalamus and by the circulating levels of T_4 and T_3. If for any reason there is a decrease in circulating levels of thyroid hormones, TSH is secreted and thyroid function is increased; if exogenous thyroid hormone is administered, TRH secretion is supressed and eventually the thyroid gland becomes inactive and regresses. It appears that the plasma concentrations of both T_4 and T_3 (and possibly intracellular formation of T_3 from T_4 in the pituitary) are important factors in the release of TSH; they also may modulate the interaction of TRH with its receptors in the pituitary (Goodman and Van Middlesworth, 1980; Hinkle and Goh, 1982; Larsen, 1982; Ross et al., 1986). Lastly, in the pituitary T_4 undergoes 5'-mono-deiodination to T_3. In the rat about 50 percent of T_3 within pituitary cells arises from this means. When serum T_4 is reduced but T_3 is normal, pituitary intracellular T_3 is reduced, and cells are able to respond to the decreased serum T_4 and increase TSH secretion (Larsen, 1982).

Thyroid hormone-responsive tissues contain a variable number of nuclear receptors for thyroid hormones (mainly T_3) usually in excess of several thousand per cell (Oppenheimer, 1979). Under euthyroid conditions in the rat, usually about 30 to 50 percent of the sites are occupied by T^3, although in the pituitary more like 80 percent of the sites are filled under physiological conditions. The T_3-receptor complex is quite labile with a half-life for dissociation of about 15 minutes; the released T_3 reenters the exchangeable cellular pool where it can complex with another receptor or exit the cell. The half-life for T_3 clearance from the plasma in experimental animals is variable, being about 6 hr in the rat (Oppenheimer, 1979).

Studies on the regulation of TSH output from the pituitary have indicated

a link between T_3 nuclear receptor occupancy and the mRNA levels for the TSH subunit chains. Administration of exogenous T_3 resulted in decreases in TSH mRNA levels in the pituitaries and in transplanted pituitary tumors of thyroidectomized mice within 1 day of administration (Chin et al., 1985). Subunit messenger RNA elongation in nuclei isolated from pituitary tumors of mice treated *in vivo* with T_3 is decreased within 1/2 hr after hormone administration, and mRNA levels were reduced within 1 hr (Shupnik et al., 1985). It appears that the decrease in mRNA is either due to decreased transcription or decreased stability of the mRNA transcripts. A straight-line relationship existed between the proportion of nuclear T_3 receptors occupied and the proportional reduction in TSH subunit transcripts in transplanted pituitary tumors (Shupnik et al., 1986). A 50 percent reduction in mRNA transcripts occurred when about 45 percent of the receptors were occupied; this occurred at plasma T_3 levels of about 1 ng/mL (1.5×10^{-9} M).

Other studies have investigated the effects of withdrawal of T_3 on TSH mRNA levels in thyroidectomized mice bearing transplanted pituitary tumors (Ross et al., 1986). Plasma T_3 levels dropped precipitously within 1 day after withdrawal; plasma TSH concentrations rose fourfold between 1 and 2 days; and tumor TSH subunit mRNA levels increased markedly between days 1 and 2.

These experiments demonstrate the rapid response of the pituitary gland to increases and decreases in plasma T_3 levels. It seems that pituitary cells modulate the levels in TSH subunit mRNAs as a function of the proportional occupancy of the numerous nuclear receptors for T_3.

IV. THYROID AND PITUITARY GLAND NEOPLASIA

As described in the previous section, the pituitary exerts a delicate control over the morphological and functional status of the thyroid, and thyroid hormones are in turn important regulators of pituitary function. It is perhaps not surprising, therefore, that the pituitary may be affected profoundly by factors causing thyroid gland dysfunction. Because of this close dependency, it is appropriate to discuss thyroid and pituitary neoplasia in the same section.

A. THYROID NEOPLASIA

While, statistically, clinical thyroid cancer is not a serious human health problem in the United States (it accounts for 0.4 percent of all cancer and 9 in 1 million deaths annually), occult thyroid cancer discovered at autopsy is much more common (average about 2 percent autopsies). Other thyroid lesions, like "nodules" noted upon palpation of the thyroid, occur in about 4 to 7 percent of adults and are of concern to physicians because they may be or develop into thyroid malignancies (Paynter et al., 1986; De Groot, 1979; Sampson et al., 1974; Rojeski and Gharib, 1985).

1. Induction

Thyroid neoplasia may be induced by exposure of experimental animals to a variety of exogenous chemicals or physical agents. This is the major focus of this paper and is discussed in some detail in Section V.

It has been recognized for some time, however, that thyroid gland follicular cell neoplasia can also be induced in experimental animals by a number of other factors that cause thyroid gland dysfunction, in particular those leading to hypothyroidism. Among these factors are iodine deficiency (Bielschowsky, 1953; Axelrod and Leblond, 1955; Schaller and Stevenson, 1966) and subtotal thyroidectomy (Dent et al., 1956). In addition, thyroid tumors can result from the transplan-

tation of TSH-secreting pituitary tumors (Dent et al., 1956; Haran-Guera et al., 1960; Sinha et al., 1965).

The one factor common to each of these conditions is that they all lead to increased production of TSH and prolonged stimulation of the thyroid gland by "excess" TSH. In the first two conditions this results from chronic stimulation of the pituitary in response to a deficiency in the circulating levels of thyroid hormones (see Section III). Also note that nothing has been given to these animals: instead the tumors developed in the absence of something that is normally present (i.e., iodine and thyroid gland mass). In the third case, excess TSH comes from the transplanted pituitary tumor. Thus, irrespective of the cause, it appears that prolonged stimulation of the thyroid-pituitary feedback mechanism that results in release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia.

Support for the role of TSH in thyroid carcinogenesis also comes from irradiation studies. X-irradiation is the only demonstrated human thyroid carcinogen. High doses of irradiation commonly associated with thyroid tumor development are associated with thyroid parenchymal cell killing and compensating increase in TSH. The types of tumors produced by irradiation are the same as those noted following purposeful manipulation of TSH (e.g., iodine deficiency). In addition, treatments which raise TSH levels cooperate with irradiation in increasing the frequency of thyroid tumors, while ablation of TSH stimulation (e.g., hypophysectomy) under these experimental conditions blocks tumor development (Doniach, 1970, 1974; Nadler et al., 1970; NAS, 1980). Thus, part of the irradiation-induced carcinogenicity appears to be due to or responsive to increases in TSH levels.

Still further support for the role of TSH in thyroid carcinogenesis comes from experiments using chemicals which reduce circulating thyroid hormone levels

and result in increases in TSH (see Section V.B.). Thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone that reestablish thyroid-pituitary homeostasis or by hypophysectomy (for examples, see Yamada and Lewis, 1968; Jemec, 1980).

2. Morphological Stages in Thyroid Neoplasia

The progressive morphological changes that occur in thyroid tissues in response to prolonged elevated levels of TSH have been studied in some detail and are qualitatively similar irrespective of the nature of the stimulus causing TSH elevation (low iodine diet, goitrogen exposure, etc.) (Gorbman, 1947; Denef et al., 1981; Philp et al., 1969; Santler, 1957; Wynford-Thomas et al., 1982a; Wollman and Breitman, 1970). Following initiation of long-term TSH stimulation, changes in the thyroid exhibit three different phases--an initial lag phase of several days, a period of rapid growth, and a period of declining growth rate as a plateau is attained.

During the lag or latent period, that may last for several days, thyroid weight and DNA content remain relatively constant. Rapid changes occur in the morphology of the gland during this period, however, characterized by resorption of colloid from the follicular lumen and by increases in epithelial cell volume (the cells change from a cuboidal to a more columnar form) and vascularity. Consequently, the latent period is characterized by a redistribution of thyroid tissue and compartment volumes and particularly by hypertrophy of the follicular epithelial cells.

With continued TSH stimulation, the latent period is followed by a rapid and prolonged increase in thyroid weight and size. Although all thyroid tissue components proliferate to some extent, the major changes observed are associated with follicular cell hyperplasia. Thus, there are dramatic increases in both mitotic activity and in the number of follicular cells per gland (Wynford-Thomas

et al., 1982a). There are, however, limits to the extent to which thyroid hyperplasia, as well as thyroid weight and size can continue to increase. Thus, despite a sustained TSH stimulus (e.g., administration of goitrogen) and sustained increases in the circulating levels of TSH, mitotic activity of the follicle cells progressively declines, and thyroid size and weight level off to a plateau (after about 80 days of goitrogen treatment) (Wynford-Thomas et al., 1982a, b). If the TSH stimulus is withdrawn for 25 days and then reintroduced, the maximum size of the thyroid remains unchanged (Wynford-Thomas et al., 1982b). Although far from definitive, the mechanism of this "desensitization" to the stimulating effects of TSH does not appear to be due to a significant "downregulation" (decrease) of the number of TSH receptors per cell (Witte and McKenzie, 1981; Davies, 1985). While subsequent studies (Wynford-Thomas et al., 1982c; Stringer et al., 1985) have failed to elucidate the desensitization mechanism, it has been suggested that it is mediated by an intracellular change in the follicular cell either at the receptor or postreceptor level. Clearly, there exists an intracellular or intercellular control mechanism that limits the mitotic response of thyroid follicle cells to TSH, which led Wynford-Thomas et al. (1982c), to propose that the failure of this control mechanism might be the first step in neoplasia. Possibly thyroid cells undergoing repeated cell division become irreversibly committed to a differentiated state and are no longer able to respond to TSH. On the other hand, cellular responsiveness to TSH may depend upon interactions with other growth mediators. In support of this, TSH-induced increases in cell number in vivo were closely correlated with changes in receptor density for another protein growth factor (somatomedin A) (Polychronakos et al., 1986).

Certainly, under experimental conditions of prolonged stimulation by TSH, diffuse thyroid hyperplasia may progress to a nodular proliferation of the

follicular cells and eventually to neoplasia (Gorbman, 1947; Money and Rawson, 1950; Griesbach et al., 1945; Doniach and Williams, 1962). While many of the resulting tumors are benign, prolonged and excessive thyroid stimulation may result in malignant tumors. The morphology of thyroid tumors in laboratory rodents has been discussed in several reviews (Doniach, 1970b; Boorman 1983; Frith and Heath, 1983). Studies with humans show a similar morphologic progression of the thyroid up through nodular hyperplasia and "adenomatous" lesions following prolonged stimulation by TSH (Ingbar and Woeber, 1981; see Section VI. of this paper)

3. Reversibility of Morphological Progression to Thyroid Cancer

Several important questions arise concerning the progression of the different morphological states towards thyroid cancer, particularly with respect to the extent to which the progression is reversible. Thus, it is important to know at what point (if any) and by what mechanism, the progression through hypertrophy, hyperplasia, nodule formation, and neoplasia becomes irreversibly committed to the formation of a malignant tumor. Undoubtedly, the final answer to these and other questions will have to await a more thorough understanding of the molecular biology of the complex events resulting in thyroid neoplasia (see Section IV.C.).

There is ample experimental evidence, however, showing that, to a significant though unknown extent, the morphological progression towards thyroid malignancy can be halted and at least partially reversed by removing the source of, and/or correcting for, the excessive thyrotropic stimulation. This may be achieved by administering adequate amounts of thyroid hormones to hypothyroid animals (Purves, 1943: Bielschowsky, 1955; Furth, 1969; Paynter et al., 1986) or by effecting surgical hypophysectomy (Astwood et al., 1943; MacKenzie and MacKenzie, 1943; Nadler et al., 1970). Goiters in persons living in iodine-deficient areas

tend to reverse following introduction of iodine in persons with hyperplasias of short duration (Ingbar and Woeber, 1981; see Section VI. of this paper). In each case, these procedures counter the effect of the source of TSH stimulation.

The extent to which morphological progression in the thyroid can be reversed, however, clearly depends on the extent to which the process has progressed i.e., the severity and particularly the duration of the insult causing TSH stimulation. On cessation of long-term goitrogen treatment or replacement of a long-term, low-iodine diet with a high-iodine diet, the size and weight of the thyroid typically decrease. If the pathological process has not progressed too far (e.g., hyperplastic goiter) regression may be complete (Gorbman, 1947; Greer et al., 1967; Ingbar and Woeber, 1981). There is even one report that propylthiouracil-induced cellular proliferation (including metastasis to the lung) regressed to normal when goitrogen administration to animals was stopped (Dunn, 1975). In the same study propylthiouracil-stimulated thyroid tissue transplanted into other animals did not continue to proliferate and retain its tumorigenic status unless the animals were treated with propylthiouracil. Others have pointed out the need for ongoing TSH stimulation in the perpetuation of "hyperplastic-neoplastic" thyroid lesions either in the animals where the lesions arose or in hosts receiving transplants of the material (Todd, 1986: see Doniach, 1970b).

In contrast, little or no indication of morphological reversibility was observed when rats that had received up to 500 ppm ethylene thiourea in their diets for a period of 2 years were returned to a control diet (Graham et al., 1973). In another study (Bielschowsky and Goodall, 1963) methylthiouracil-induced thyroid lesions in the mouse continued to progress after goitrogen administration was stopped and replaced by thyroid hormone treatment. Most other studies indicate varying degrees of reversibility following discontinuation of goitrogen

administration (Arnold et al., 1983; Wollman and Breitman, 1970; Wynford-Thomas et al., 1982c) or return of animals from a low-iodine to a high-iodine diet (Greer et al., 1967).

In humans it has been common practice to use high doses of thyroid hormone to try to suppress the growth of thyroid "nodules" and help differentiate non-neoplastic from neoplastic growths (Rojeski and Gharib, 1985). The idea is that preneoplastic lesions would regress upon cessation of TSH stimulation brought about by the added hormone. Although variable success in reducing nodule size has been noted in the past, a recent, carefully done study failed to show any treatment-related reductions (see study and review, Gharib et al., 1987). Thus the role of TSH in maintaining the size of human thyroid nodules and their potential for reversal upon cessation of TSH stimulation requires further investigation.

Typically, the reversal is marked by a reduction of thyroid gland size and weight beginning a few days after removal of the TSH stimulus, and this is associated with a loss of DNA indicating a decrease in the number of cells present; some of this seems to be due to a reduction in the number of follicular cells (Wollman and Breitman, 1970; Wynford-Thomas et al., 1982c). The mechanism by which cells are lost from the thyroid may be cell death or migration. Regression is associated with involution of the thyroid that involves a decrease in vascular dilation, a marked diminution of follicular cell size and shape (from columnar to cuboidal) and a return of follicular colloid material (Gorbman, 1947). These qualitative changes in thyroid histology almost always occur following the removal of the TSH stimulus. However, if the goiter has been present for several weeks, or months, the thyroid gland continues to remain at least two to three times its normal size and weight despite a return to its normal histological appearance (Greer et al., 1967: Wollman and Breitman, 1970; Wynford-Thomas et al., 1982c).

B. PITUITARY NEOPLASIA

Following chronic iodine deficiency (Axelrod and Leblond, 1955), treatment with goitrogens (Griesbach, 1941; Griesbach et al., 1945) or surgical or ^{131}I-induced thyroidectomy (Doniach and Williams, 1962; Carlton and Gries, 1983), the anterior pituitary frequently exhibits a loss of acidophilic cells, an increase in basophil cells, and develops swollen "thyroidectomy cells" some of which contain cytoplasmic granules. These cells contain TSH (Osamura and Takayama, 1983) and, in the eyes of some researchers, may progress to TSH-secreting adenomas (Furth et al., 1973; Bielschowsky, 1955), although other authors have failed to demonstrate tumors in such treated animals (for instance, see Ohshima and Ward, 1984, 1986). Pituitary hyperplasia and neoplasia appear to result from the same treatments causing thyroid neoplasia--conditions leading to prolonged thyroid hormone decrease and excessive secretion of TSH by the pituitary gland.

C. MOLECULAR CONSIDERATIONS IN THYROID CARCINOGENESIS

Any hypothesis developed to explain the mechanism for carcinogenesis must be consistent with what is known about the specific type of cancer and the physiological and biochemical system in which it develops. Animal experiments have clearly shown that increased levels of TSH are associated with development of thyroid hyperplasia and, later, with thyroid neoplasia. These end points, hyperplasia and neoplasia, manifest two processes that are going on in the thyroid: one is an increased commitment to cell division, which leads to hyperplasia; the other is the transformation of normal cells into neoplastic cells. Recent work at the cellular level indicates that induction of cell division (which can lead to hyperplasia) and the transformation of normal to altered (neoplastic) cells is the result of a complex interaction of different

cell systems. For thyroid follicular carcinogenesis, it appears that TSH is a major component in these interactions.

It is generally recognized that, under normal conditions, the control of cell division requires the interaction of a number of endogenous factors which work through a number of common pathways; exogenously added materials may also have profound effects on this system. It seems there are at least two such control steps centered in the pre-DNA synthetic part of the cell cycle, and TSH is one of the factors operating there in thyroid cells. Certain protein growth factors which operate through receptors on the cell surface are other stimuli that influence cell division. In a similar manner, the transformation of normal cells into an altered state with neoplastic potential also seems to be dependent upon the interaction of different factors. TSH may also play an active role here.

This section reviews available molecular information about the control of cell growth in thyroid cells and their conversion to neoplastic cells, and attempts to incorporate this information into a plausible mechanistic framework. Figure 4 illustrates a not fully satisfactory, but hopefully instructive, hypothetical model for the interaction of TSH and other factors in inducing cell proliferation and transformation in the thyroid gland leading to neoplasia. Although there are gaps in the understanding of the processes involved, what is known about the thyroid is consistent with the existing understanding of the components involved with the control of mammalian cell division. It is also consistent with current thinking that carcinogenesis is a multistep process and that multiple factors may influence its course. And finally, it accords special weight to TSH as playing a significant role in cell proliferation and in carcinogenesis of the thyroid gland.

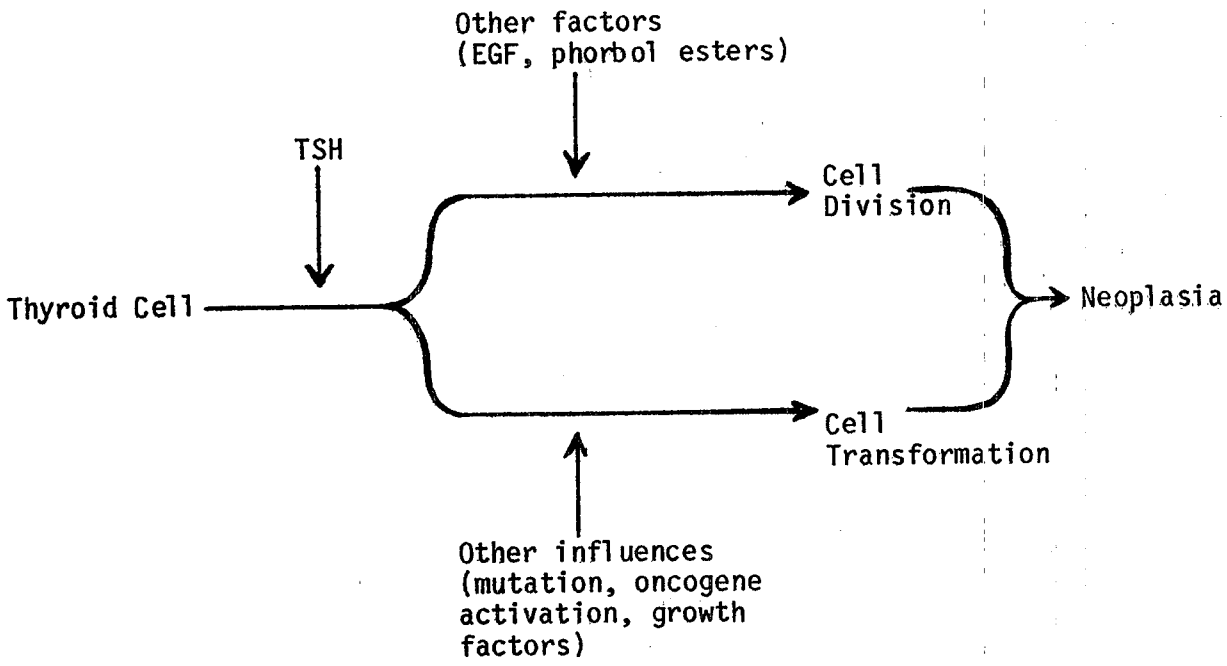


Figure 4. Hypothetical model for thyroid carcinogenesis.

1. Stimulation of Cell Division

a. Influence of TSH--TSH interaction with its receptor on the surface of the thyroid cell results in activation of adenyl cyclase and resultant production of cAMP, the activation of the phosphatidylinositol pathway, commencement of certain thyroid-specific differentiated functions that result in the formation of the thyroid hormone and stimulation of cell division. Although all cultured cells do not respond to TSH alone by increasing cell division (murine and canine do; porcine, ovine, and human do not [see Saji et al., 1987]), the following steps have been identified in those that do respond. Almost immediately (within 15 to 30 minutes) after addition of TSH to quiescent thyroid cells in culture, there are marked increases in the levels of the mRNAs for the cellular protooncogene, c-fos. A similar pattern is found for transcripts of the proto-oncogene, c-myc, but the induction is delayed somewhat, with the peak occurring at about 1 to 2 hr after TSH addition. These effects of TSH can be mimicked by direct addition of cAMP analogs or other factors that increase cellular cAMP (Dere et al., 1985; Tramontano et al., 1986a; Colletta et al., 1986). Interestingly, human thyroid adenomas and carcinomas are characterized by c-myc expression, which is not found in the surrounding normal thyroid tissue. In addition, like normal cells in culture, adenoma cells respond to TSH in a dose-related manner by increasing the levels of c-myc transcripts (Yamashita et al., 1986). This finding in human cells is in contrast to that cited above (Saji et al., 1987).

The protein products of the c-fos and c-myc protooncogenes are thought to play a role in the replication of cells. Both c-myc and c-fos code for proteins that are largely restricted to the cell nucleus and appear to be functionally linked to DNA synthesis. The latter is illustrated by experiments showing that when monoclonal antibody to human c-myc protein is added to isolated nuclei, there is an inhibition of DNA synthesis and replicative DNA polymerase activity;

the inhibition can be overcome by the addition of excess c-myc protein (Studzinski et al., 1986).

There is additional evidence to indicate that oncogene expression may be an important factor in triggering cell division. For instance, certain human cancers have been shown to have chromosome rearrangements involving c-myc. This relationship has been well established for cases of Burkitt lymphoma (B-cell cancer) (Taub et al., 1982; ar-Rushdi et al., 1983; Nishikura et al., 1983) and to a lesser extent for certain T-cell leukemias (Erikson et al., 1986; Finger et al., 1986) It is thought that chromosomal translocations move c-myc to the regulatory units of immune response genes in these cells and bring about constitutive activation of the oncogene which then provides a continued stimulus for cell proliferation (see review by Croce, 1986).

TSH also seems to affect to some extent the phosphatidylinositol pathway within cells (Kasai and Field, 1982; Tanabe et al., 1984; Bone et al., 1986) which is a major transduction system of signals across cell membranes (see Nishizuka, 1986 and next section) as is the cAMP system. Just how this effect of TSH may influence thyroid cell division has not yet been determined.

b. Other Factors--Experiments in a number of cell systems have identified control points in the pre-DNA synthetic part of the cell cycle which must be passed for cells to replicate DNA and go into cell division. For instance, mammalian cells treated with one chemical stimulus (e.g., platelet-derived growth factor which is known to stimulate c-myc) did not commence DNA synthesis until other substances were added to the medium (Stiles et al., 1979; Smeland et al., 1985). Current investigations on the interaction of various factors in the control of cell division have been summarized by Goustin et al. (1986) and Rozengurt (1986).

Work with thyroid cells also indicates that a number of growth factors and

cell systems are operating which influence a cell's commitment to cell division. For illustrative purposes, emphasis here will be placed on three of these: epidermal growth factor, the protein kinase c system (see Table 1), and the somatomedins.

Epidermal growth factor (EGF) is a naturally occurring polypeptide present in a number of organs that binds to specific receptors on sensitive cells. This binding results in activation of receptor-associated tyrosine kinase which phosphorylates the EGF receptor and other sites and helps to bring about its cellular action. EGF is present in adult tissues; a related growth factor, transforming growth factor type α, is present in neoplasms and embryonic tissues and may be an embryonic form of EGF. It is interesting to note that one of the viral oncogenes, v-erbB, is a mutation of the EGF receptor gene where the binding-site portion of the receptor has been deleted, and that this mutation may result in constitutive activation resulting in continued cell proliferation (Goustin et al., 1986).

There is some work that indicates that EGF plays a role in the regulation of cellular activity and cell division in thyroid cells in culture. Its role in vivo needs to be ascertained. Unlike TSH, EGF blocks certain differentiated functions that typify thyroid action, such as formation of thyroglobulin by thyroid cells in culture (Westermark et al., 1983; Bachrach et al., 1985; Roger et al., 1986). In in vivo studies, infusion of sheep over a 24-hour period with EGF resulted in a profound drop in serum T_4 and T_3 which started within 10 hours after commencing administration. Part of this reduction in circulating thyroid hormones appears to be due to their enhanced metabolism (Corcoran et al., 1986). These authors cite other work which show that thyroid hormone administration results in increased tissue levels and urinary excretion of EGF. It thus seems that some feedback exists between levels of EGF and thyroid hormones.

TABLE 1. EFFECTS OF STIMULI ON THYROID CELLS

Stimulus	Enzyme activity	Induces c-fos & c-myc	Stimulates cell division	Effect on differentiated functions	Other
TSH	adenyl cyclase	+	+	Enhances	Enhances EGF binding to its receptor
EGF	tyrosine kinase	?	+	Inhibits	
TPA[a]	protein kinase c	?	+	Inhibits	Inhibits EGF binding to its receptor and tyrosine kinase activity

[a]TPA, 12-O-tetradecanoylphorbol 13-acetate, a phorbol ester.

EGF also produces increases in thyroid cell division in thyroid cells. By about one day after addition of EGF to thyroid cells in culture, there is stimulation in DNA synthesis (Westermark et al., 1983; Roger et al., 1986), as was seen after administration of TSH. TSH increases the binding of EGF to its receptor on thyroid cells and, in combination with EGF, enhances DNA synthesis above that seen with EGF alone (Westermark et al., 1986).

Another cell-surface related mechanism results in the activation of protein kinase c. It is generally recognized that this system is one of the major information-transferring mechanisms from extracellular to intracellular sites in many cells throughout the body (see review by Nishizuka, 1986). Receptor binding of a host of biologically active substances (e.g., hormones, neurotransmitters) is followed by hydrolysis of inositol phospholipids along two paths: one leads to calcium mobilization, the other to activation of protein kinase c. The kinase transfers phosphate groups to various proteins which results in a modulation of their action. Many studies have demonstrated that certain tumor promoters in the two-stage mouse skin carcinogenesis model, including the phorbol esters, can bind to cell receptors and activate protein kinase c (see Nishizuka, 1986).

Phorbol esters, like EGF, inhibit differentiated thyroid cell functions and stimulate cell division. As in other cells (Friedman et al., 1984), phorbol esters increase protein kinase c activity and block EGF binding of its receptor in thyroid cells (see Table 1) (Bachrach et al., 1985; Ginsberg and Murray, 1986; Roger et al., 1986). It is not known if EGF and phorbol esters stimulate expression of the c-fos and c-myc protooncogenes in the thyroid, although there is some evidence for this in mouse 3T3 cells (Kruijer et al., 1984; Muller et al., 1984; Kaibuchi et al., 1986).

A series of polypeptide substances related to insulin and termed somatomedins (insulin-like growth factors, IGFs), are known to exist which help to control cell growth in numerous tissues (see Goustin et al., 1986). Concentrations of somatomedins in the blood are regulated by growth hormone. They are produced by the liver and almost all organs of the body, seemingly the products of mesenchymal cells (Han et al., 1987). Although they may or may not stimulate DNA synthesis in cells when they are the only added factor, they frequently interact significantly with other growth factors in bringing about cell division (Stiles et al., 1979).

In cultured rat thyroid cells very high concentrations of insulin alone will induce cells to replicate DNA (Smith et al., 1986). It was hypothesized, then demonstrated, that this effect was most likely due to cross reactivity of insulin with the somatomedin C (IGF-I) receptor (Tramontano et al., 1986b, 1987: Saji et al., 1987). In rat thyroid cells TSH and somatomedin C (or insulin) synergize in inducing DNA synthesis, but are additive in regard to increasing cell growth (Tramontano et al., 1986b); such DNA-replication synergy was not noted in porcine cells (Saji et al., 1987).

Although studies on thyroid cells indicate that TSH, EGF, phorbol esters, and somatomedin C (and insulin) can each stimulate cell division in cultured thyroid cells, it does not mean that these factors are the only ones. For instance, many of the culture systems used in these studies included serum, which is known to have a number of growth factors in it. In other cases, the culture medium was supplemented with hormones, growth factors, and other substances (e.g., somatostatin, cortisol, transferrin) which are known to effect cell cycle traverse (Bachrach et al., 1985; Colletta et al., 1986; Westermark et al., 1983).

c. Possible Controls in Thyroid Cell Division--As discussed earlier, it appears that the control of cell division in mammalian cells is in the pre-DNA synthetic portions of the cell cycle. By using combinations of substances, two control points have been identified; both points must be passed for cells to commence DNA replication. Although there are significant differences in response among cell systems, factors that seem to affect the first regulatory point include such things as platelet-derived growth factor and the c-fos and c-myc oncogenes, whereas those operating at the second control point include somatomedin C, EGF, and the c-ras oncogene (Stiles et al., 1979; Leof et al., 1982; see Goustin et al., 1986). Since TSH is also known to activate adenyl cyclase and c-fos and c-myc expression in thyroid cells (Dere et al., 1985; Colletta et al., 1986; Tramontano et al., 1986a), it seems possible that it may act at the first control point. This is supported by the observation that combinations of TSH with EGF or somatomedin C lead to enhanced DNA synthesis in thyroid cells (EGF and somatomedin C are putative second control step agents) (Westermark et al., 1986; Tramontano et al., 1986b, 1987).

The placement of the protein kinase c system in the control of thyroid gland cell division is uncertain, since its effect on cell proliferation is not enhanced by either TSH or EGF. As indicated previously, phorbol ester administration to thyroid cells diminished EGF binding to its receptor (Bachrach et al., 1985). It also appears that TSH itself may increase the phosphatidylinositol pathway in addition to affecting cAMP (Bone et al., 1986). On the other hand, the protein kinase c and adenyl cyclase systems often play complementary roles in mammalian cells to enhance cell division and other functions (Nishizuka, 1986; Rozengurt, 1986). More information is needed in this area.

Insulin (and related substances) seem to play a facilitating role in the thyroid. Alone in high concentrations it can induce thyroid cells in medium

without serum to synthesize DNA, and it enables TSH to enhance this effect (Wynford-Thomas et al., 1986). Insulin is active at both control points in certain mouse 3T3 cells as well (Rozengurt, 1986).

A model can be constructed for control of cell division in the thyroid gland (Figure 5) that includes the two pre-DNA synthetic steps. The model engenders the known effects of various factors on thyroid cells, and reflects certain observations in other mammalian cell systems. Although the model is not fully satisfactory, due to the inconsistencies across cell systems, it depicts certain interactions that may exist in the thyroid gland and suggests possible future research directions.

2. Cellular Transformation

As with the control of cell division, complex interactions among different factors seem to be operating during the transformation of normal to altered cells with neoplastic potential. Although activation of a single oncogene is not sufficient to produce transformation, activation of two different oncogenes is a common means of transforming cells (see reviews by Weinberg, 1985; Barbacid, 1986). Frequently the cooperation includes an oncogene whose product is localized to the nucleus (e.g., c-fos, c-myc) with one whose product is in the cytoplasm (e.g., c-ras, c-src). As was mentioned previously, nuclear oncogenes can be activated by chromosomal translocation of the oncogene to cellular regulatory sequences; other activation mechanisms include the insertion of viral regulatory segments next to the nuclear oncogene, gene amplification (increase in the number of copies of the oncogene per cell), and stabilization of the oncogene gene product. On the other hand, cytoplasmic oncogenes tend to be activated by point or chromosomal mutations which affect the structure of their gene products (Weinberg, 1985).

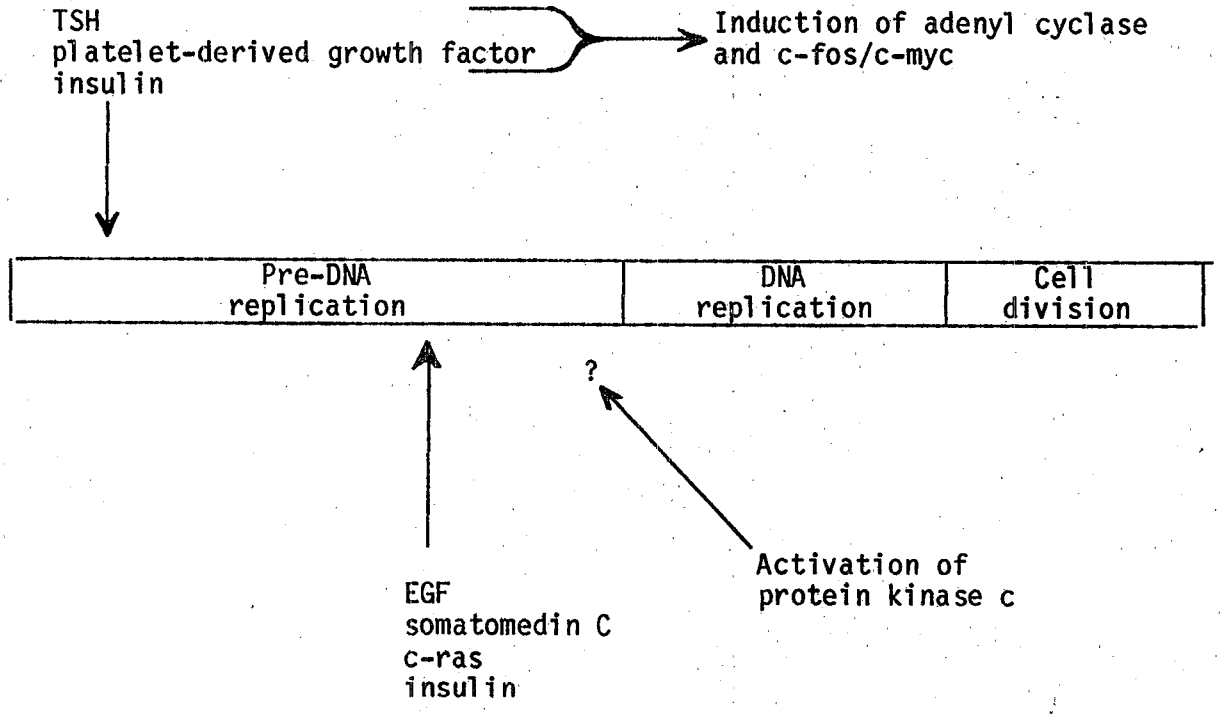


Figure 5. Possible control points for cell division in the pre-DNA synthetic portion of the cell cycle.

TSH enhances c-fos and c-myc expression that may in turn interact with other factors in bringing about cell transformation. If the stimulus for TSH secretion from the pituitary is long-term, as in the case of continued exposure to an antithyroid substance, it seems possible there could be continued oncogene transcription and a continued emphasis on cell proliferation which could result in hyperplasia. Still other stimuli (e.g., activation of a second oncogene, certain point or structural mutations, interplay with growth factors) may aid in the transformation process and bring about neoplasia.

This hypothesis is consistent with recent studies which indicate that c-myc may be a necessary component in cellular transformation, but that it is not sufficient in itself to bring about the condition. Studies of transgenic mice support this conclusion (Adams et al., 1985; Langdon et al., 1986). Combinations of the DNA of c-myc and the enhancer region of the Eu-immunoglobulin locus were made and injected into fertilized mouse eggs which were transplanted into maternal hosts. The DNA became incorporated into the cells of the body of the developing organism (transgenic recipients). Within a few months after birth, almost all animals developed malignant B-cell lymphomas and died. It seems that during development there is constitutive expression of c-myc with a great expansion of multiple clones of B-cell precursors. However, only one clone develops into a tumor, and this seems to occur at variable times during development. This has led the authors to propose that although c-myc expression favors proliferation of B-cell precursors, some genetic event, like activation of a second oncogene, may be required for transformation to malignancy.

Studies on the thyroid gland are consistent with the idea that c-myc (through TSH stimulation) may interact with other stimuli in bringing about cell transformation. For instance, an enhancement of the carcinogenic response is noted when a treatment that increases TSH (e.g., iodide deficiency) follows

application of a genotoxic agent (e.g., irradiation, nitrosamine) (see Section IV) which might produce a mutation that activates a second oncogene or some other effect.

One is still faced, however, with the observation that treatments that ensure prolonged TSH stimulation, as have been discussed previously, lead to neoplasia. Three possibilities exist: (1) TSH simply enhances spontaneously occurring events (e.g., mutations in regulatory sequences like oncogenes). The finding of thyroid neoplasms in about 1 percent of some untreated laboratory animals (Haseman et al., 1984) is in keeping with the idea that "spontaneous mutations" might exist in control animals that might predispose animals for development of thyroid tumors. (2) Through its effect on cell division, TSH may expand the thyroid cell population at risk for a spontaneous event and then promote neoplasia once a spontaneous mutation occurs. (3) TSH alone, via some yet undisclosed mechanism, might produce cellular transformation.

V. EXOGENOUS FACTORS INFLUENCING THYROID-PITUITARY CARCINOGENESIS

The observations presented in the previous section demonstrated that prolonged increases in TSH output are associated with thyroid cellular hypertrophy and hyperplasia and, finally, with neoplasia in the absence of exogenously added agents. This section summarizes known information on thyroid carcinogenesis following application of exogenous stimuli. In the main, it, too, shows the important role of chronic TSH stimulation in thyroid carcinogenesis. Information on physical and chemical agents affecting thyroid-pituitary physiology and carcinogenesis is summarized. Chemical classes associated with thyroid tumors in the NCI/NTP animal studies are listed, and analyses are conducted on the specific chemicals from those classes as to their antithyroid activity and genotoxicity.

A. PHYSICAL FACTORS

External ionizing radiation is a known thyroid carcinogen in humans and experimental animals (NAS, 1980). Internal radiation, following administration of ^{131}I (a β - and a γ-radiation emitter) produces thyroid tumors in animals, but the evidence in humans from the follow-up of treated Graves' disease patients is less firmly established (NAS, 1980; NCRP, 1985; see Becker, 1984). A recent paper purports the hypothesis that radioiodines may account for thyroid nodules following the detonation of a hydrogen bomb in the Marshall Islands in the Pacific Ocean (Hamilton et al., 1987). Although irradiation can alter DNA and induce mutation and, thus, influence thyroid carcinogenesis via genotoxic mechanisms, others have speculated that the follicular cell damage induced by irradiation may impair the gland's ability to produce thyroid hormone and, thus, places the thyroid under conditions of long-term TSH stimulation.

B. CHEMICAL FACTORS

1. Goitrogens

Early interest in naturally occurring chemicals causing thyroid enlargement arose from observations that rabbits fed diets composed mainly of cabbage leaves frequently developed goiters (Chesney et al., 1928). Similar observations were subsequently made with two purified synthetic chemicals (sulfaguanidine and 1-phenyl-2-thiourea) during nutritional/physiological studies with rats (Mackenzie et al., 1941; Richter and Clisby, 1942). When it was realized that the primary action of these and related compounds was to inhibit synthesis of the thyroid hormones, their potential therapeutic value in hyperthyroidism became evident.

a. Naturally-occurring (dietary) substances--These materials have been reviewed in detail by VanEtten (1969). The early observations of goiters in rabbits maintained on cabbage-leaf diets (Chesney et al., 1928) were followed by the discovery that the seeds of rape and other brassica species (cabbage, brussels sprouts, turnips, and mustard) also contained substance(s) that were goitrogenic when incorporated into rat diets (Hercus and Purves, 1936; Kennedy and Purves, 1941). Prolonged dietary exposure to rape seed led to the development of adenomatous goiters (100 percent in 27 months) in rats (Griesbach et al., 1945). L-5-Vinyl-2-thiooxazolidone (goitrin) has been identified as the active goitrogen in turnips and the seed and green parts of other cruciferous plants. Goitrin from these sources may be passed to humans in the milk of cows feeding on such plants. In humans, goitrin appears to be about as active as propylthiouracil (Haynes and Murad, 1985). Peanuts are also reported to be goitrogenic in rats (Srinivasan et al., 1957), the active component being the glucoside, arachidoside.

b. Synthetic compounds--Synthetic chemicals exhibiting goitrogenic activity may be divided into three major structural groups: thionamides, aromatic amines, and polyhydric phenols. The synthetic goitrogens are discussed briefly below, but have been extensively reviewed by Cooper (1984) and Paynter et al. (1986).

(i) Thionamides. These include derivatives of thiourea and heterocyclic compounds containing the thioureylene group. The latter includes most of the compounds (e.g., propylthiouracil, methimazole, and carbimazole) used therapeutically for hyperthyroidism in humans. Among the many chemicals in this group, one nitrogen atom may be replaced by oxygen or sulfur; however, the thionamide group is common to all. Other active compounds in this class are derivatives of imidazole, oxazole, thiazole, thiadiazole, uracil, and barbituric acid. The naturally occurring goitrin, present in cruciferous plants, also belongs to this group of compounds.

(ii) Aromatic amines. Examples of compounds of this type are the sulfonamides, sulfathiazole, and sulfadiazine (Haynes and Murad, 1985). Optimal antithyroid activity of this group of compounds is associated with a para-substituted aminobenzene structure with or without aliphatic (e.g., methyl) substitution on the amino nitrogen. It is of interest that several methylene- and oxydianilines (and alkyl substituted derivatives) have also been shown to possess goitrogenic activity (Hayden et al., 1978) and like, the sulfonamides, to increase thyroid neoplasms in rats (Weisburger et al., 1984).

(iii) Polyhydric phenols. The antithyroid activity (hypothyroidism and goiter) of resorcinol was first observed following the use of this material for treatment of leg ulcers in humans (Haynes and Murad, 1985). Subsequent studies have established that antithyroid activity is associated with compounds with meta-polar-substituents on the benzene ring. Thus, hexyresorcinol, phloroglucinol, 2,4-dihydroxybenzoic acid, and meta-aminophenol are active, whereas catechol,

hydroquinone, and pyrogallol are not (Paynter et al., 1986).

c. Modes of Action--Antithyroid agents belonging to structural groups i, ii, or iii all exert their activity by direct interference with the synthesis of the thyroid hormones in the thyroid gland. All appear to block the incorporation of iodine into tyrosyl residues of thyroglobulin and by inhibiting the coupling of the idotyrosyl residues into idothyronines. It was proposed by Taurog (1976) that the antithyroid agents inhibit the enzyme peroxidase that is responsible for the conversion of iodide to the iodinating species and the subsequent iodination and coupling of the tyrosyl residues. This has been confirmed by subsequent studies (Davidson et al., 1978; Engler et al., 1982) showing that the compounds bind to and inactivate peroxidase when the heme of the enzyme is in the oxidized state. It is likely that these compounds show some inhibitory selectivity towards the different peroxidase-catalyzed reactions (i.e., iodination vs. coupling) (Haynes and Murad, 1985). There is also evidence that some of the compounds (e.g., propylthiouracil) inhibit the peripheral deiodination of T_4 to T_3 (Geffner et al., 1975; Saberi et al., 1975).

Because of their ability to inhibit thyroid hormone synthesis, all of the above compounds have the potential to reduce circulating levels of T_4 and T_3 and, consequently, to induce the secretion of TSH by the pituitary. As a result, prolonged exposure to such compounds can be expected to induce thyroid gland hypertrophy and hyperplasia and ultimately may lead to neoplasia.

2. Enzyme inducers

In addition to chemicals exerting effects directly at the thyroid, as was summarized in the previous section, a number of others acting at peripheral sites can cause equally profound disturbances in thyroid function and morphology. Of particular interest are those compounds that induce hepatic and/or extrahepatic enzymes responsible for the metabolism of many endogenous and exogenous compounds.

These chemicals can increase the metabolism of thyroid hormone, result in a reduction in circulating thyroid hormone, and stimulate an increase in TSH. Following long-term exposure to these agents, the thyroid gland undergoes hypertrophy and hyperplasia and finally, neoplasia.

a. Foreign compound metabolism and enzyme induction--

i. General. The enzymes responsible for the metabolism of foreign compounds constitute a remarkably diverse group of proteins that catalyze a variety of reactions associated with either the primary (Phase I) metabolic attack on a chemical (oxidation, reduction, hydrolysis) or with its subsequent secondary (Phase II) metabolism (e.g., conjugation with glucuronide, sulfate, amino acids, and glutathione) (Testa and Jenner, 1976). The enzymes are associated with the endoplasmic reticulum or cytosol of the liver and a number of extrahepatic tissues. The enzymes serve an important functional role in increasing the polarity, water-solubility, and excretability of the vast majority of fat-soluble foreign compounds and often result in a decrease in their biological activity or toxicity. Because of the latter, they are frequently referred to as detoxication enzymes (Wilkinson, 1984).

ii. Induction. Enzyme induction refers to the phenomenon whereby exposure of an animal to a given foreign compound results in the enhanced activity through de novo synthesis of a spectrum of the enzymes involved in Phase I and Phase II metabolism (Conney, 1967). Induction typically results in an increase in the rate at which the inducer and other compounds are metabolized and excreted.

Since the enzymes responsible for foreign-compound metabolism are thought by many to have evolved as a biochemical defense against potentially harmful environmental chemicals (Wilkinson, 1984), induction may be viewed as a biological adaptation that can provide important short-term benefits for survival. On the other hand, in the light of increasing evidence that the enzymes detoxifying

one chemical may activate another (Cummings and Prough, 1983), there has been concern that enzyme induction may represent a mechanism through which potentially dangerous toxicological interactions can occur following chemical exposure.

Another cause for some concern is that several of the enzymes that participate in foreign-compound metabolism are also known to play important roles in the metabolism of physiologically important endogenous chemicals such as hormones. Clearly, any changes in the levels of enzymes responsible for the synthesis or breakdown of such compounds could lead to physiological imbalances with potentially serious consequences (Conney, 1967).

iii. Different inducer types. Inducers of the enzymes involved in foreign-compound metabolism have been divided into at least two different categories on the basis of their characteristic effects on cytochrome P-450 and monooxygenase activity (Mannering, 1971; Lu and West, 1978; Ryan et al., 1978; Lu and West, 1980). One of these, typified by phenobarbital, led to a significant increase in liver size and weight and caused the substantial proliferation of hepatic endoplasmic reticulum. Induction was associated with increases in cytochrome P-450 and a large number of monooxygenase reactions that enhanced metabolic (oxidative) capability towards many foreign compounds. The spectrum of oxidative reactions induced is now known to result mainly from the induction of one major isozyme of cytochrome P-450 that, in rats, is referred to as cytochrome P-450b (Ryan et al., 1978). A large number of drugs and other foreign compounds including the chlorinated hydrocarbon insecticides (DDT and its analogues and the cyclodienes like chlordane and aldrin) exhibit induction characteristics similar to phenobarbital and are generally referred to as "PB-type" inducers.

Early studies with the polycyclic hydrocarbon, 3-methyl cholanthrene (3MC), clearly indicated that the induction characteristics of this compound were quite distinct from those of PB (Mannering, 1971). In contrast to the

latter, treatment of animals with 3MC did not cause large increases in liver size or in the proliferation of endoplasmic reticulum; neither did it result in large increases in cytochrome P-450. Instead, 3MC resulted in the formation of a qualitatively different form of cytochrome P-450, known generally as cytochrome P-448 and now referred to in rats as cytochrome P-450c (Mannering, 1971; Lu and West, 1978; Ryan et al., 1978). This cytochrome is associated with a rather limited number of oxidative reactions, the best known of which is aryl hydrocarbon hydroxylase (AHH) (Ryan et al., 1978; Eisen et al., 1983; Conney, 1982). AHH has received a lot of attention in recent years because of its role in the metabolic activation of compounds like benzo[a]pyrene to potent carcinogens (Eisen et al., 1983; Conney, 1982). Inducers of the "3MC-type" include a number of polycyclic aromatic hydrocarbons, naphthoflavone, and several halogenated dibenzo-p-dioxins; 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most effective inducer of this type to be discovered (Poland and Glover, 1974). The mechanism of action of inducers of this type involves high affinity binding to a cytosolic receptor and subsequent migration of the inducer-receptor complex to the nucleus where the transcriptional effect leading to enhanced protein synthesis is initiated (Eisen et al., 1983). Induction of this type is genetically controlled by the so-called Ah locus in rodents and, while the true identity of the cytosolic receptor remains unknown, it is hypothesized to be a receptor for some hormone or other physiologically important ligand.

While the "PB-type" and "3MC-type" inducers still constitute the two major categories of inducers, it is now recognized that a number of other types exists, each characterized by increased levels of a distinct spectrum of isozymes of cytochrome P-450 and other enzymes. It is also apparent that a number of compounds share some of the characteristics of more than one group and cannot be strictly classified. Technical mixtures of polyhalogenated biphenyls (PCBs

and PBBs), for example, exhibit characteristics of both PB- and 3MC-type inducers (Alvares et al., 1973), probably due to the presence in the mixtures of a number of isomers representing each type.

In addition to inducing a characteristic spectrum of isozymic forms of cytochrome P-450, many of the inducers also result in enhanced titers and activities of other enzymes involved in foreign-compound metabolism. While these have not been well documented, they include epoxide hydratases, glutathione (GSH)-S-transferases and several of the transferases (UDP-transferases, sulfotransferases) associated with secondary conjugation reactions (Jacobsen et al., 1975; Lucier et al., 1975; Ecobichon and Comeau, 1974). It has been suggested that, like cytochrome P-450, these enzymes may also exist in multiple isozymic forms and that different inducers may enhance the activity of specific isozymes with a characteristic range of substrate specificities.

b. Metabolism of thyroid hormones--The liver not only constitutes a target tissue for the thyroid hormones but is also an organ responsible for the metabolic inactivation of the hormones and their elimination from the body. About half the T_4 elimination from the body of the rat occurs via the bile, whereas in humans only about 10 to 15 percent is lost in this way (Oppenheimer, 1987). While there appear to be quantitative differences in the relative rates of elimination of T_4 and T_3, it is probable that both are excreted by a qualitatively similar mechanism. The major pathway of elimination involves conjugation of the phenolic hydroxyl group of T_4 with glucuronic acid and biliary excretion of the resulting glucuronide (Figure 1) (Galton, 1968; Bastomsky, 1973); sulfate conjugates may also be produced and excreted. On entering the intestine a portion of the conjugate may undergo hydrolysis by intestinal bacteria to release free thyroid hormone that may be reabsorbed into the circulation; this process is referred to as enterohepatic circulation. Unhydrolyzed conjugate

cannot be reabsorbed and is excreted in the feces (Houk, 1980).

c. Effect of inducers on thyroid function and morphology--

(i) PB-type inducers. Initial reports on the goitrogenic effects of a number of PB-type inducers in both birds and rodents began to appear in the mid- to late 1960s. Modest to substantial increases in thyroid weight were reported in rats treated with phenobarbital (Japundzic, 1969; Oppenheimer et al., 1968) and isomers of DDD (Fregly et al., 1968), in pigeons treated with p,p'-DDE (Jefferies and French, 1969), p,p'-DDE or dieldrin (Jefferies and French, 1972) and in bobwhite quail exposed to p,p'-DDT or toxaphene (Hurst et al., 1974). Chlordane, another chlorinated hydrocarbon, enhanced thyroid function and caused hepatic accumulation of ^{125}I-T_4 in rats (Oppenheimer et al., 1968). Histological examination of the thyroids of treated animals typically showed a reduction in follicular colloidal material and increased cellular basophilia and hyperplasia (Fregly et al., 1968; Jefferies and French, 1972), and it was noted by several workers that these changes were similar to those occurring in response to increased circulating levels of TSH. Support for the effect being a response to increased TSH, rather than a direct effect on the thyroid, is found in studies demonstrating that the goitrogenic response of the thyroid to phenobarbital could be prevented by hypophysectomy or the administration of T_4 (Japundzic, 1969).

The effects of PB-type inducers on thyroid function are now known to be quite complex and to involve a number of factors relating to the distribution, tissue binding, metabolism, and excretion of thyroid hormones. Animals treated with phenobarbital show increased hepatocellular binding of T_4 combined with enhanced biliary excretion of the hormone (Oppenheimer et al., 1968; 1971). In intact rats, these changes simply result from an increased rate of turnover of T_4 that is compensated by release of TSH and enhanced thyroidal secretion of

new hormone. As a result, no change in serum protein-bound iodine (PBI) is observed following treatment with phenobarbital (Oppenheimer et al., 1968). In thyroidectomized rats, however, phenobarbital reduces serum PBI and also reduces the hormonal effects of administered T_4 (Oppenheimer et al., 1968; 1971) The ability of phenobarbital to reduce circulating levels of exogenously supplied T_4 in a human hypothyroid patient has been reported. The major factors leading to enhanced turnover of T_4 in animals treated with PB-type inducers seem to be increased hepatocellular binding due mainly to proliferation of the endoplasmic reticulum (Schwartz et al., 1969) and a modest increase in bile flow that enhances the overall rate of biliary clearance (Oppenheimer et al., 1968). Phenobarbital (Oppenheimer et al., 1968) and DDT (Bastomsky, 1974) cause only minimal increase in biliary T_4 excretion, and in rats treated with DDD isomers, fecal excretion of ^{131}I-T_4 was not observed until 24hr. after hormone treatment (Fregly et al., 1968). While DDT slightly enhanced the proportion of biliary ^{125}I present as T_4-glucuronide, neither PB nor DDT (Bastomsky, 1974) are reported to have significant effects on the rate of glucuronidation of T_4.

Several studies have been conducted on the effects of PB-type inducers on thyroid hormone status in healthy human volunteers or in patients on different drug regimens. Drugs studied include phenobarbital, carbamazepine, rifampicin, and phenytoin (diphenylhydantoin). Most of the studies report decreased serum levels of T_4 (both protein-bound and free) (Rootwelt et al., 1978; Faber et al., 1985; Ohnhaus and Studer, 1983), but reports vary on the changes observed in serum levels of T_3 and rT_3 depending on the type and concentration of the inducer employed. Ohnhaus and Studer (1983) observed a relationship between increasing levels of microsomal enzyme induction and decreasing serum levels of T_4 and rT_3 in healthy volunteers treated with combinations of antipyrine and rifampicin. An effect was only observed, however, at induction levels that

decreased the half-life of antipyrine by more than 60 percent. Induction of hepatic enzymes is apparently only one of several mechanisms through which diphenylhydantoin can reduce circulating levels of T_4 (Smith and Surks, 1984). Other possible mechanisms by which diphenylhydantoin might act include serum protein displacement of the thyroid hormones, effects on the binding and biological activity of T_3, and even effects on hypothalamic and pituitary regulation of TSH. Despite significantly decreased serum levels of T_4, there seem few reports of humans being placed in a hypothyroid condition as a result of treatment with drugs that induce liver microsomal enzyme activity. An exception is the observation that persons being maintained on exogenously supplied thyroid hormone become hypothyroid when given diphenylhydantoin or phenobarbital unless their thyroid hormone doses are changed (Oppenheimer, 1987). Furthermore, TSH levels never change significantly from those observed in the controls.

(ii) 3MC-type inducers. The effects on the thyroid of 3MC-type hepatic enzyme inducers (polycyclic aromatic hydrocarbons, TCDD, etc.) are perhaps the best understood of the compounds under discussion. A major mechanism involved seems to be the induction of the T_4-UDP-glucuronyl transferase that constitutes the rate-limiting step in the biliary excretion of T_4 (Bastomsky, 1973). The effect is particularly well illustrated with reference to a variety of thyroid hormone parameters 9 days after treatment of rats with a single dose of 25 ug/kg TCDD (Bastomsky, 1977a). Biliary excretion of ^{125}I (during the first hour after injection of ^{125}I-T_4 and the biliary clearance rate of plasma ^{125}I-T_4 were increased about 10-fold. Somewhat unexpectedly, the biliary excretion of T_3 was unaffected by TCDD. As a direct consequence of these changes in metabolism and excretion, serum T_4 concentrations (but not those of T_3) were reduced to half those in controls. Other workers have reported decreased serum T_4 concentrations following TCDD treatment (Potter et al., 1983; Pazdernik and Rozman, 1985;

Rozman et al., 1985). TCDD treatment also elevated serum concentrations of TSH and, as a result, produced thyroid goiters (measured by elevated thyroid weight) and enhanced ^{131}I uptake by the thyroid. There are conflicting reports as to whether TCDD enhances bile flow (Bastomsky, 1977a; Hwang, 1973) but this does not seem to be a major factor in its goitrogenic action.

While TCDD is an unusually potent inducer of UDP-glucuronyl transferases, it appears to be at least somewhat similar to compounds such as 3MC (Bastomsky and Papapetrou, 1973; Newman et al., 1971), 3,4-benzo[a]pyrene (Goldstein and Taurog, 1968), and the polychlorinated and polybrominated biphenyls (PCBs and PBBs) (see below) all of which have been shown to enhance the biliary excretion of T_4 at least partly by increasing the formation of T_4-glucuronide. TCDD did not uniformly increase hepatic UDP-glucuronyl transferase activity towards all substrates; it enhanced activity towards p-nitrophenol but not towards testosterone or estrone. Its effect on the T_4-transferase does not seem to have been investigated.

Recently, some investigators have suggested that the explanation for the interactions of TCDD with thyroid hormone levels is that T_4 and TCDD have common molecular reactivity properties that might allow them to react with the same receptors (McKinney et al., 1985a, b). Indeed, McKinney and his co-workers consider that many of the toxic effects of TCDD result directly from its action as a thyroxine agonist. This theory contrasts with the views of Poland's group (Poland and Knutsen, 1982) that TCDD toxicity segregates with the Ah locus and involves TCDD binding to the cytosolic receptor. Moreover, McKinney's views are not consistent with recent experimental results (Potter et al., 1986), and the entire area requires more research attention.

(iii) Mixed-type. Perhaps as a result of their widespread contamination of the environment and their well documented occurrence in human foods, the

toxicological properties of PCBs and PBBs have received considerable attention (Kimbrough, 1974).

Daily feeding of commercial mixtures of PCB (Arochlors) or PBB (Firemaster) to rats (5, 50, and 500 ppm) led to striking dose- and time-dependent histological changes in thyroid follicular cells (Collins et al., 1977; Kasza et al., 1978). These changes included increased vacuolization and accumulation of colloid droplets and abnormal lysosomes with strong acid phosphatase activity in follicle cells. Microvilli on the lumen surface became fewer in number, shortened and irregularly branched, and Golgi bodies were smaller; at higher exposures mitochondria were swollen with disrupted cristae. It has been suggested that the combined presence of an abnormally large number of colloid droplets and lysosomes in the follicle cells might indicate interference with the normal synthesis and/or secretion of thyroid hormones (e.g., cleavage of active thyroxine from thyroglobulin). PBB has been found to accumulate preferentially in the thyroid following 20 days of treatment and was still present 5 months after administration (Allen-Rowlands et al., 1981). Sequestration of PBB in the thyroid might indicate binding to thyroidal macromolecules, and it has been suggested that PBB might interfere with the organification of iodide by peroxidase. More work in this area is needed.

Instead of comprising a single layer of cuboidal or low columnar epithelium, the follicular cells of PCB-treated animals became more columnar with multiple layers and hyperplastic papillary extensions into the colloid. Similar follicular cell hyperplasia has been reported in other chronic (Norris et al., 1975) and subchronic studies (Sleight et al., 1978) with PBBs. The histological changes, which are similar to those observed in animals treated with TSH (Seljeld et al., 1971), were accompanied by substantially decreased (>three-fold) serum thyroxine levels in PCB-treated rats (Collins et al., 1977). Residual effects were

observed 12 weeks after termination of exposure, probably reflecting the persistent nature of the PCBs. However, it is important to note that, even in animals exposed to the highest doses of PCBs, both the histological and functional abnormalities were reversible and were minimal 35 weeks after cessation of treatment.

The search for a mechanistic explanation of PCB- or PBB-induced thyroid hyperplasia has focused on the biochemical events occurring on exposure to these compounds. Direct effects on the thyroid cannot be discounted, and recent evidence suggests that disturbances in thyroid hormone synthesis and distribution may occur following long-term administration (Byrne et al., 1987). More work is needed in this area. However, most attention has been given to peripheral effects that modify the distribution, metabolism, and excretion of thyroid hormones and as a consequence may cause thyroid hyperplasia indirectly through activation of the normal feedback mechanism involving TSH. Thyroid parameters changed following short-term oral or cutaneous administration of PCBs to rats have been extensively studied by Bastomsky and co-workers (Bastomsky, 1974, 1977b; Bastomsky and Murthy, 1976; Bastomsky et al., 1976) and include:

(a) Increased biliary excretion (about five fold) and bile:plasma ratio (about 12-fold) following injection of ^{125}I-T_4.

(b) Increased biliary clearance rate of plasma ^{125}I-T_4 more than 20-fold.

(c) Modest increase in bile flow (less than two fold).

(d) Decreased total serum and free T_4 concentrations.

(e) Increased ^{131}I uptake by thyroid.

It is apparent from these data that PCBs have effects that are similar to both "PB-type" and "3MC-type" inducers. PCBs are reported to be potent inducers of liver T_4-UDP-glucuronyl transferase (Bastomsky and Murthy, 1976) and, as with the "3MC-type" inducers such as TCDD, this undoubtedly accounts, at least

partially, for the increased biliary excretion of T_4. On the other hand, PCB also displaced the thyroid hormones from their binding proteins in the serum (Bastomsky, 1974; Bastomsky et al., 1976), an effect usually associated more with "PB-type" compounds. Because of its PB-like activity, it is also possible that PCB enhances hepatic binding of T_4. It may be a combination of the induction of T_4-UDP-glucuronyl transferase and the displacement from serum binding proteins that lead to such high bile:plasma ratios of T_4 following PCB treatment; much smaller T_4 bile:plasma ratios are observed with compounds like salicylate that effect displacement but not enzyme induction (Osorio and Myant, 1963). Conversely, the effects of changes in binding proteins on metabolism of thyroid hormone under steady-state conditions do not seem to have been studied, and at least some arguments can be mounted that would suggest that no change in metabolism would occur under those conditions.

PCBs are reportedly quite specific in their ability to selectively induce different isozymes of UDP-glucuronyl transferase. Thus, in addition to inducing the glucuronidation of T_4, the PCB-induced isozyme(s) will also enhance activity towards p-nitrophenol (Ecobichon and Comeau, 1974) and 4-methylumbelliferone (Grote et al., 1975); PCB did not enhance the glucuronidation of bilirubin, however (Bastomsky et al., 1975).

The effects of PCB treatment on circulating levels of T_3 are clearly different from those of T_4. It has been suggested that since T_3 is more active than T_4 and because it is generated peripherally by 5'-monodeiodination of T_4, T_4 may be serving simply as a prohormone. It is now generally accepted, however, that T_4 does have intrinsic hormonal activity. It is of considerable interest to note that, in contrast to the case with T_4, treatment of rats with PCB does not result in any marked change in total serum or free concentrations of T_3. While this may result from a number of different factors (Bastomsky et al., 1976),

no completely satisfactory explanation has yet been proposed. There is some suggestion that the relatively constant circulating levels of T_3 might be due to enhanced thyroidal secretion and enhanced peripheral conversion of T_4 or T_3 in response to the PCB-induced hypothyroidism.

In summary, in addition to possible direct effects on the thyroid, mixed-type inducers such as the PCBs and PBBs have several effects that, either alone or in combination, reduce circulating levels of the thyroid hormones and cause the pituitary to release TSH. These are:

(a) Induction of T_4-UDP-glucuronyl transferase,

(b) Displacement of T_4 from serum proteins, and

(c) Increase in bile flow.

3. Other chemicals and treatment combinations

In addition to those chemicals that act directly upon the thyroid gland to inhibit the synthesis of thyroid hormone or act distal to that site to enhance thyroid hormone metabolism and removal from the body (see Section VI.B. for some other agents active in humans), there is a small group of compounds that have produced thyroid tumors in experimental animals that do not share these characteristics. Also, several investigations have indicated that combined-treatment regimens are associated with thyroid carcinogenic responses in excess of that produced by either single treatment alone.

a. Other chemicals--A few compounds have been identified that produce thyroid tumors that are not known to influence thyroid-pituitary status (see Hiasa et al., 1982), two of which are N-nitroso compounds. Rats given eight injections of N-methyl-N-nitrosourea (NMU) over a 4-week period developed thyroid tumors by week 36 without any development of goiter (Tsuda et al., 1983). Likewise, there was no evidence of diffuse follicular hyperplasia in rats given a single dose of NMU and observed at 33 weeks, even though some animals had thyroid

neoplasms (Ohshima and Ward, 1984). In a similar way, N-bis(2-hydroxypropyl)-nitrosamine (DHPN) administration for 8 weeks led to thyroid tumors by 20 weeks without any increase in thyroid weight (Hiasa et al., 1982); this observation was confirmed in a second laboratory (Kitahori et al., 1984). Both nitrosamines produce tumors at sites other than the thyroid.

The nitrosamines are a notorious group of compounds as to their potential to produce carcinogenic effects in multiple species following metabolism to reactive intermediates. Many are genotoxic in multiple test systems for different end effects.

b. Combined-Treatment Studies--Although goitrogenic stimuli that increase TSH levels (e.g., amitrole, phenobarbital, iodine deficiency) are known to induce thyroid hyperplasia and neoplasia alone, many experiments have demonstrated an enhancement of the neoplastic response when these treatments are combined with other exposures. Thus, when animals are first exposed to genotoxic physical agents (i.e., ^{131}I or X-rays) or chemical substances (e.g., certain nitroso compounds, 2-acetylaminofluorene) followed by a goitrogenic stimulus, carcinogenic responses (e.g., incidence of tumor-bearing animals, multiplicity of tumors per animal, incidence of malignancies, and tumor latency) are greater than following single treatments alone (see Appendix A).

Some have likened this response in the thyroid to the initiation-promotion (two-step) phenomena originally described for mouse skin. In that case, treatment with the first agent (initiator) confers a permanent change in cells, such that exposure (usually prolonged) to the second agent (promoter) results in neoplasms; reversal of treatments is ineffective as to tumor production. Over time it has become generally recognized that carcinogenesis is a multistep process that usually includes an initiation step as well as a promotional phase (OSTP, 1985).

The thyroid combined-treatment studies are consistent with the concepts of

initiation-promotion. The genotoxic agent might permanently alter the thyroid cell so that its accentuated growth under a goitrogenic stimulus would result in neoplasms. Also consistent with this notion is the finding that the effect of the initial treatment in the thyroid is long-lived. Rats can be treated with 4-methyl-2-thiouracil (promoter) after intervals of time at least up to 18 weeks after exposure to 2-acetyl-aminofluorene (initiator) and still go on to show an enhanced neoplastic response (Hall, 1948). On the other hand, protocols employing treatment with the "promoter" before the "initiator" have not been conducted for the thyroid. Thus, the correspondence of effects in the thyroid to those in the classical two-stage model are not established, (although they are testable).

c. Summary--Both physical and chemical agents have been implicated in thyroid carcinogenesis. Ionizing radiation remains the only confirmed carcinogenic agent for the human thyroid, an observation corroborated in experimental animals. Laboratory research has demonstrated that many substances can directly interfere with the synthesis of thyroid hormone (e.g., certain inorganic substances, thionamides, aromatic amines). Under conditions of reduced thyroid hormone levels, the pituitary increases TSH stimulation of the thyroid, which leads to a predictable set of responses including cellular hypertrophy and hyperplasia, nodular hyperplasia, and, finally, neoplasia. Pituitary tumors are also sometimes increased, seemingly due to the increased pituitary stimulation resulting from lowered circulating thyroid hormone levels.

Direct thyroidal effect is not the only way chemicals produce reductions in circulating thyroid hormone. Enzyme inducers increase the removal of thyroid hormone from the blood which, in turn, results in stimulation of the pituitary gland to secrete more TSH. The result, again, of long-term exposure is hypertrophy, hyperplasia, and eventually neoplasia. Only a limited number of chemicals have

produced thyroid follicular tumors in animals in the absence of some antithyroid effect.

C. STRUCTURE-ACTIVITY RELATIONSHIPS

1. Chemicals producing thyroid neoplasms in animals

One means of testing hypotheses concerning the mechanism of follicular cell thyroid carcinogenesis is to review those chemicals known to produce such neoplasms in experimental animals. The NCI/NTP data base is a valuable source of information because it consists of about 300 chemicals that have been subject to a somewhat standard protocol in certain strains of rats and mice. Although about half the chemicals tested have shown neoplastic effects at one or more anatomical sites, only 21 chemicals have been associated with the development of follicular cell neoplasms of the thyroid (Table 2).

These 21 compounds were not representative of the spectrum of classes of chemicals that were tested in the bioassays. Instead there was an overabundance of chemicals in structural classes that are known to influence thyroid hormone status. Over half of them (13 of 21) are either thionamides (3) or aromatic amines (10), two chemical classes that have often been linked with antithyroid activity primarily due to peroxidase inhibition. The bulk of the remaining chemicals (7 of 21) are complex halogenated hydrocarbons; members of this class are often inducers of microsomal enzymes, and at least some are known to increase the clearance of thyroid hormone from the blood. The remaining chemical, an organophosphorous compound, is not from a group typically linked to effects on the thyroid. Thus, in 20 of 21 instances, there is some basis to think that thyroid neoplasms may be related to a reduction in thyroid hormone with concomitant increase in pituitary stimulation of the thyroid through TSH.

Although most compounds producing thyroid neoplasms are members of specific chemical classes, not all members of those groups have been shown to produce

TABLE 2. CHEMICALS IN THE NCI/NTP BIOASSAY PROGRAM SHOWING AT LEAST SOME EVIDENCE OF THYROID FOLLICULAR CELL NEOPLASIA

1. Thionamides

N,N'-dicyclohexylthiourea
N,N'-diethylthiourea
trimethylthiourea

2. Aromatic Amines

a. Single ring

3-amino-4-ethoxyacetanilide
o-anisidine hydrochloride
2,4-diaminoanisole sulfate
HC Blue No. 1

b. Bridged double rings

4,4'-methylenebis(N,N-dimethyl)benzenamine
4,4'-methylenedianiline dihydrochloride
4,4'-oxydianiline
4,4'-thiodianiline

c. Miscellaneous

C.I. Basic Red 9 monochloride
1,5-naphthalenediamine

3. Complex Halogenated Hydrocarbons

aldrin
chlordane
chlorinated paraffins (C_{12}, 60% chlorine)
decabromodiphenyl oxide
2,3,7,8-tetrachlorodibenzo-p-dioxin
tetrachlorodiphenylethane (p,p'-DDD)
toxaphene

4. Organophosphous Compounds

azinphosmethyl

such tumors. For instance, among the thionamides tested by NCI/NTP, N,N'-dicyclothiourea, N,N'-diethylthiourea and trimethylthiourea yielded positive thyroid effects whereas several others did not (see Table 3).

It, therefore, seems reasonable to postulate that while a thionamide structure increases the chance that a chemical will produce thyroid tumors in long-term animal tests, structure alone is not sufficient in itself to generate such activity. The same is true for certain aromatic amines (see Section V.C.2.b.).

2. Antithyroid activity and thyroid carcinogenesis

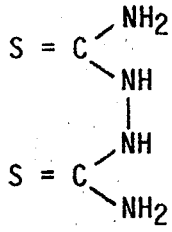
Given that many of the chemicals producing thyroid tumors in the NCI/NTP series come from chemical classes known to produce antithyroid effects by inhibition of thyroid peroxidase, a review was made of specific thionamides and aromatic amines to see if antithyroid activity was a prerequisite for thyroid carcinogenic activity. The hypothesis was borne out for the thionamides and at least some of the aromatic amines.

Generally, the criteria for selecting the specific chemicals required that they had been (1) tested for animal carcinogenicity (NCI/NTP or IARC review), and (2) evaluated for antithyroid activity. However, in some cases a chemical had been studied for carcinogenicity, but not antithyroid activity. In those cases, structurally related compounds that had been tested for antithyroid activity were chosen to act as surrogate indicators of a compound's antithyroid potential.

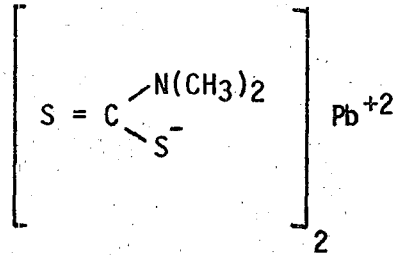
Antithyroid activity has been measured for a number of chemicals in rats and, to some extent, in humans. For rats, chemicals were administered orally at different doses for 10 days. Iodine concentrations in the thyroid were measured, and from the dose-response curve the dose that reduced the iodine concentration to a standard level was estimated (EDc). For comparison, the dose of thiouracil (a well-studied antithyroid agent) that reduced iodine

TABLE 3. THIONAMIDES NEGATIVE FOR THYROID NEOPLASIA IN NCI/NTP STUDIES

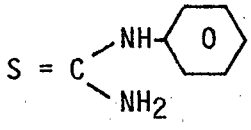
1. 2,5-dithiobiurea



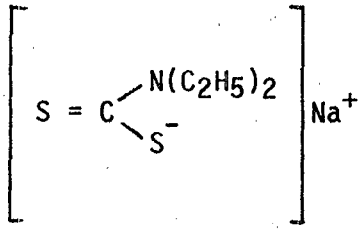
2. lead dimethyldithiocarbamate



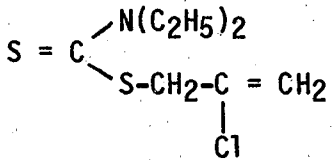
3. 1-phenyl-2-thiourea



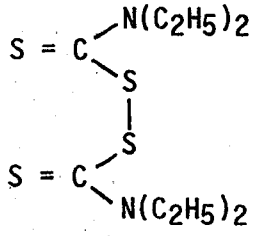
4. sodium diethyldithiocarbamate



5. sulfallate



6. tetraethylthiuram disulfide



concentration to the same level was also estimated (EDt). Antithyroid activitiy was expressed as the ratio of the estimated dose of thiouracil relative to that for the chemical (EDt/EDc), where thiouracil (in this review) is given a value of 100 (Astwood et al., 1945; McGinty and Bywater, 1945a, b).

For humans, antithyroid activity for a chemical was again measured against the effects of thiouracil (value = 100 for this review) (Stanley & Astwood, 1947). Subjects were given ^{131}I by mouth, and iodine in the thyroid was monitored externally by Geiger-Muller measurement. After 1 to 2 hours, the chemical was given orally, and the influence of the agent on the further time-course uptake of radioactivity into the gland was evaluated. The degree to which accumulation was affected was graded depending upon the completeness and duration of inhibition. Usually chemicals were studied at two or more doses.

(a) Thionamides--For the heterocyclic thionamides there is strong support for the premise that there may be a correlation between a chemical's ability to induce thyroid tumors and its ability to inhibit significantly iodine localization in the thyroid of rats and humans (Table 4A). For the thiourea-like thionamides (Table 4B), namely thiourea, trimethylthiourea, and N,N'-diethylthiourea, relative antithyroid activities of about 10 or more were associated with thyroid tumor induction. In keeping with a correlation between these effects, 2,5-dithiobiurea and tetraethylthiuram disulfide (with its structural analogue, tetramethylthiuram disulfide) both lacked antithyroid activity and did not produce thyroid neoplasia.

On the other hand, two other chemicals in the series of thiourea-like compounds need clarification. In the case of 1-phenyl-2-thiourea, a relative antithyroid value of 14 was found in rats, but the long-term NCI study in rats and mice was negative for thyroid tumors or thyroid hyperplasia. There was an absence of any toxic manifestations in dosed rats in the longterm study and a

TABLE 4A. THIONAMIDES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENICTY

HETEROCYCLIC COMPOUNDS

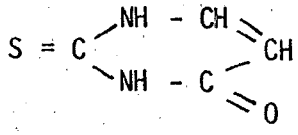
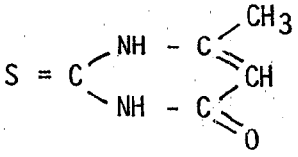
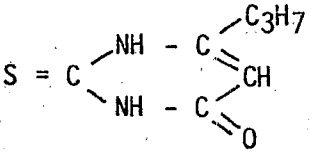
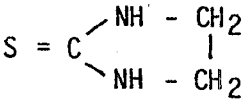
		Relative Anthithyroid Activity (thiouracil = 100)		Neoplasms[a/]		
		rat	human[c/]	thyroid[f/]		other sites
		ABH[b/]		rat	mouse	
1.	2-thiouracil	100	100	+	+	mouse-liver
						
2.	6-methylthiouracil	100	100	+	+	mouse-liver and pituitary
						
3.	6-n-propylthiouracil	1100	75	+	+	mouse-pituitary
						
4.	ethylene thiourea	40	50	+	d/	mouse-liver
						

TABLE 4B. THIONAMIDES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENICTY

THIOUREA DERIVATIVES

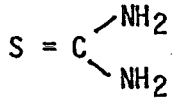
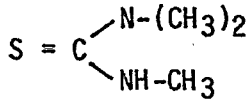
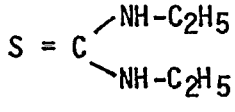
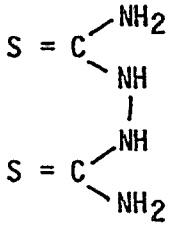
		Relative Antithyroid Activity (thiouracil = 100)			Neoplasms [a]		
		rat		human [c]	thyroid [f]		other sites
		ABH [b]	MB [e]		rat	mouse	
1.	thiourea	12	9	100	+	+	rat-liver, head, face mouse-skull
							
2.	trimethylthiourea	10	n	n	+	-	-
							
3.	N,N'-diethylthiourea	40	47	n	+	-	-
							
4.	2,5-dithiobiurea	1	n	n	-	-	-
							

TABLE 4B. (continued)

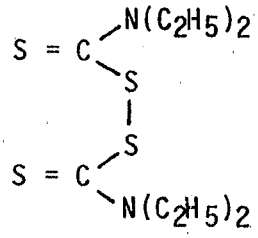
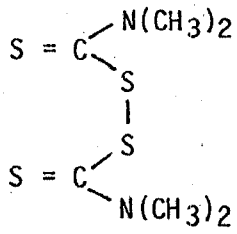
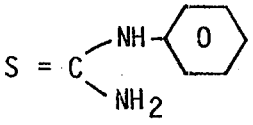
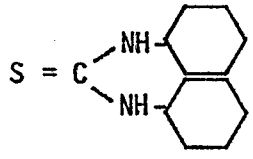
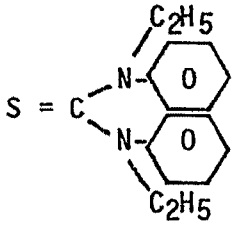
	Relative Antithyroid Activity (thiouracil = 100)			Neoplasms[a]		
	rat[b]		human[c]	thyroid[f]		other sites
	ABH	MB[e]		rat	mouse	
5. tetraethylthiuram disulfide	n	n	n	-	-	-
						
6. tetramethylthiuram disulfide	1	n	n	n	n	n
						
7. 1-phenyl-2-thiourea	n	14	n	-	-	-
						

TABLE 4B. (continued)

		Relative Antithyroid Activity (thioracil = 100)			Neoplasms[a]		
		rat		human[c]	thyroid[f]		other sites
		ABH[b]	MB[e]		rat	mouse	
8.	N,N'-dicyclohexyl-thiourea	n	n	n	+	-	-
							
9.	1,3-diethyl-1,3-diphenyl thiourea	1	n	n	n	n	n
							

KEY:
a - from IARC reviews
b - Astwood et al., 1945
c - Stanley and Astwood, 1947
d - Mouse study did not examine thyroid
e - McGinty and Bywater, 1945a
f - from NCI studies, except thiourea (IARC review)
n - not tested.

question whether a maximum tolerated dose had been used. In addition, after 78 weeks of chemical administration, dosed animals were observed for an additional 26 weeks in rats and 13 weeks in mice before sacrifice. Since thyroid hyperplasia is oftentimes reversible, it is possible any lesions produced by dosing may have regressed during the observation period. Other investigators have reported thyroid hyperplasia after 6 weeks of phenylthiourea administration to rats (Richter and Clisby, 1942) indicating that the chemical may induce thyroid neoplastic effects under certain conditions. Further work on this compound may bear this out.

In the second case, N,N'-dicyclohexylthiourea showed increased incidences of thyroid follicular hyperplasia in dosed rats and mice in the NCI study, and there were some increases in follicular cell carcinomas in male rats. Although N,N'-dicylohexylthiourea has not been tested for antithyroid activity, its structural analogue, 1,3-diethyl-1,3-diphenyl thiourea failed to show significant antithyroid effects in the rat.

(b) Bridged double ring aromatic amines--Like the thionamides, certain aromatic amines with double rings attached by a simple ether-like bridge, show a correlation between antithyroid activity and thyroid carcinogenesis (Table 5). 4,4'-Methylenedianiline, 4,4'-methylenebis (N,N'-dimethyl)benzenamine and 4,4'-thiodianiline (chemicals no. 1 through 3, respectively) show both attributes, and although 4,4'-oxydianiline (no. 4) has not been tested for antithyroid activity, it has close structural similarity with the other three chemicals and also produces thyroid neoplasms. In keeping with its potential for antithyroid effects, chemical no. 4 produced increases in the number of TSH-secreting cells in the pituitary in rats following chronic administration (Murthy et al., 1985), and both chemicals no. 4 and no. 1 produced thyroid enlargements in the NCI 90-day prechronic studies. All of these observations--antithyroid activity, thyroid

TABLE 5. AROMATIC AMINES RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENESIS

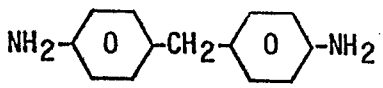
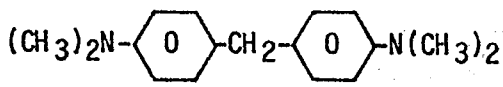
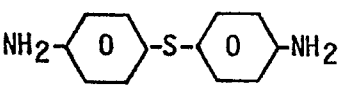
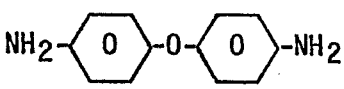
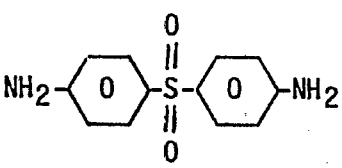
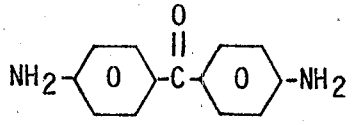
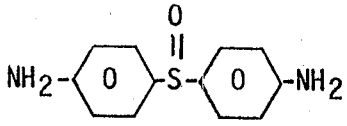
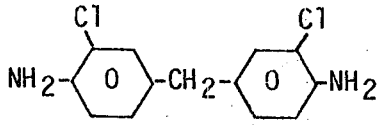
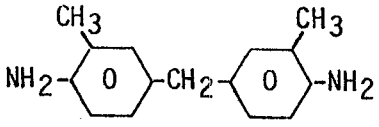
Bridged Double Ring Compounds	Relative Antithyroid Activity - rat (thiouracil =100)	Neoplasms[a/] thyroid rat	thyroid mouse	other sites
1. 4,4'-methylenedianiline dihydrochloride 	25[c/]	+	+	mouse-liver rat-liver
2. 4,4'-methylenebis (N,N-dimethyl) benzenamine 	25[c/]	+	-	mouse-liver
3. 4,4'-thiodianiline 	15[d/]	+	+	mouse-liver rat-liver
4. 4,4'-oxydianiline 	n	+	+	mouse-liver, harderian gland rat-liver
5. 4,4'-sulfonyldianiline 	4[d/]	-	-	rat-mesenchymal

TABLE 5. (continued)

	Relative Antithyroid Activity - rat (thiouracil =100)	Neoplasms[a/] thyroid rat	thyroid mouse	other sites
6. Michler's ketone	n	-	-	mouse-liver
				
7. 4,4'-diaminodiphenylsulfoxide	12[e/]	n	n	n
				
8. 4,4'-methylene bis (2-chloroaniline) [b/]	n	-	-	mouse-liver, vascular rat-liver, lung
				
9. 4,4'-methylene bis (2-methylaniline) [b/]	n	-	n	rat-liver
				

KEY: a - NCI/NTP bioassay except for last two chemicals in table
b - IARC review of carcinogenicity
c - Astwood et al., 1945
d - McGinty and Bywater, 1945b
e - McGinty and Bywater, 1946a
n - not tested

enlargement in subchronic studies, and increases in the cell types of the pituitary that secrete TSH--are consistent with the hypothesis that bridged ring aromatic amines induce thyroid neoplasms by reducing circulating thyroid hormone levels and increasing TSH.

Other compounds in this series show results that are hard to interpret. 4,4'-Sulfonyldianiline (no. 5), which has an $-SO_2-$bridge between the rings, had a low antithyroid value of 4 in rats and was negative for thyroid tumors. Compound no. 6 with a -C(O)- bridge was also negative for thyroid tumors. Although chemical no. 7, which has an -S(O)- bridge was negative for thyroid neoplasms, it was associated with an antithyroid value of 12 in the rat. Antithyroid values in the 10 to 15 range have been linked with positive thyroid tumorigenic effects for chemical no. 3 and some of the thionamides, e.g., thiourea. Further studies on antithyroid activity may help to clarify this inconsistency.

It is also interesting to note that compounds structurally identical to 4,4'-methylenedianiline (no. 1) except for substitution on the rings in the 2,2'-positions (chemicals nos. 8 and 9) are negative for thyroid tumors. It would be interesting to measure their antithyroid activity.

In summary, for both the thionamides and bridged double ring aromatic amines there appears to be support for concluding that there is a good relationship between antithyroid activity and thyroid carcinogenesis, although further work needs to be done to be able to interpret some results. It seems possible that agents that are known to inhibit thyroid hormone output may be potential thyroid carcinogens under certain experimental conditions.

(c) Characteristics of Single Ring Aromatic Amines--Many single ring aromatic amines have been evaluated for carcinogenicity in experimental systems and have shown positive effects (Clayson and Garner, 1976; Weisburger et al., 1978; see

review by Lavenhar and Maczka, 1985), but only a few of them have produced neoplasms in the thyroid. Of the single ring compounds that have been tested by the NCI/NTP (Appendix B), o-anisidine (no. 1), 2,4-diaminoanisole (no. 2), 3-amino-4-ethoxy-acetanilide (no. 3), and HC Blue No. 1 (no. 9) were the only ones to produce thyroid neoplasms. Of these agents only 2,4-diaminoanisole produced thyroid tumors in all four species-sex categories; the others produced such tumors in only one group.

The single ring aromatic amines have not been examined systematically as to their antithyroid activity; therefore, these agents cannot be analyzed as to the relationship between peroxidase inhibition and thyroid carcinogenesis. However, from a preliminary review of structural analogues that have been tested for carcinogenicity (Appendix B), there is little indication that specific ring substitutions are influencing thyroid carcinogenic potential.

3. Genotoxicity and Thyroid Carcinogenesis

It has been generally accepted by the scientific community that mutagenesis plays a role in carcinogenesis. In the case of thyroid follicular cell tumors, however, it has been suggested that a hormonal feedback mechanism involving increased output of thyroid stimulating hormone from the pituitary gland in response to low thyroid hormone levels may be operating (Woo et al., 1985; Paynter et al. 1986). Even though hormone imbalance may play a role in thyroid carcinogenesis, it is important also to evaluate the mutagenic potential of agents causing these tumors.

This section explores the relationship between the induction of thyroid neoplasms in rodents and their outcome on several short-term tests of genotoxicity. If the hypothesis that TSH plays a significant role in thyroid carcinogenesis is true, one might expect that chemicals producing thyroid tumors in experimental animals would not show genotoxic potential in any predictable way. If, instead,

thyroid carcinogenesis were largely due to chemical reactivity and not to hormonal derangement, then thyroid carcinogens might be genotoxic agents.

This review largely draws upon those compounds that were tested in rats and mice for carcinogenicity by the NCI/NTP and produced thyroid neoplasms. Structurally related compounds that did not produce thyroid tumors are included for comparison. The genotoxicity data on these chemicals are from the NTP, much of which has not been published in peer-reviewed journals and at least some of which could be considered preliminary in nature.

Chemicals are divided into structural classes: thionamides, aromatic amines, and halogenated hydrocarbons. The NTP short-term test data on many compounds are limited and, therefore, are hard to interpret. In order to get a better appreciation of the spectrum of genotoxic effects that may occur among members of a chemical class, two compounds, ethylene thiourea and 4,4'-oxydianiline, were considered in detail (using the open literature) as examples of thionamides and aromatic amines, respectively. An example of the halogenated hydrocarbon class was not included, since members of this group generally show little indication of genotoxic potential. A third compound, amitrole, was also included for detailed review; it does not belong to any of the above chemical classes, but it is recognized as being an inhibitor of thyroid peroxidase as are certain thionamides and aromatic amines.

(a) Thionamides--For the three chemicals tested by NCI/NTP that were positive for thyroid tumors, the existing information gives little indication of significant genotoxic potential (Table 6). Of 14 chemical-test comparisons on these agents for both gene mutation and chromosomal effects, there are only two positive responses. There appears to be slightly more positive genotoxicity data in the case of thionamides that tested negative for thyroid follicular cell tumors (10 of 19 tests) than for those that tested positive. However, no firm conclusions

TABLE 6. GENOTOXICITY DATA FOR THIONAMIDES

	GENE MUTATIONS			CHROMOSOMAL EFFECTS	
	SA	ML	SLRL	CA	SCE
1. Chemicals Positive for Thyroid Tumors					
N,N'-Dicyclohexylthiourea	-	-	n	-	+
N,N'-Diethylthiourea	-	+	-	-	-
Trimethylthiourea	-	-	-	-	-
2. Chemicals Negative for Thyroid Tumors					
1-Phenyl-2-thiourea	-	u	n	+	+
2,5-Dithiobiurea	-	n	n	-	+
Tetraethylthiuram disulfide	-	+	n	+	-
Sulfallate	+	n	n	n	n
Lead dimethyldithiocarbamate	+	u	-	+	+
Sodium diethyldithiocarbamate	-	+	n	-	-

Symbols: SA, Salmonella reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in Drosophila; CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; -, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

can be made from this limited data set.

The genotoxicity of ethylene thiourea, a compound known to produce thyroid tumors, was assessed in greater detail (see Appendix C). Although it was concluded from the journal articles that there is evidence for genotoxicity when ethylene thiourea is supplemented with sodium nitrite (*Salmonella* with metabolic activation, *in vivo* cytogenetics, dominant lethal, micronucleus), presumably via the formation of *N*-nitrosoethylene thiourea, there is much less evidence for the genotoxic potential of ethylene thiourea itself. The compound shows little indication of gene mutation activity: negative to weakly positive effects in bacteria, negative in *Drosophila*, and conflicting information in yeast and mammalian cells in culture (negative in CHO cells and divergent results in mouse lymphoma cells). Chromosomal effects are not demonstrated in cells of higher eukaryotes in culture or in vivo. DNA damage tests showed conflicting results in bacteria, yeast, and human cells in culture.

In contrast to the effects listed above, several thionamides are positive for *in vitro* transformation. Thiourea, *N*,*N*'-dicyclohexylthiourea, and ethylene thiourea have shown positive effects in Syrian hamster cells (SHE and BHK), and the first two also transformed rat embryo cells (Rauscher murine leukemia virus-infected) (Heidelberger et al., 1983; Styles, 1981; Daniel and Dehnel, 1981). However, these three chemicals and *N*,*N*'-diethylthiourea were reported negative in simian adenovirus-7 infected Syrian hamster and rat cells (Heidelberger et al., 1983).

In sum, the lack of genotoxic effects noted with the thionamides that produced thyroid tumors in the NCI/NTP studies is borne out by the detailed review of ethylene thiourea. There is little indication of gene mutation or chromosomal effects. There are conflicting results with the DNA damage tests and *in vitro* transformation.

(b) Aromatic amines--Unlike thionamides, the class of aromatic amines commonly demonstrates genotoxic effects for both point mutations and chromosomal effects (Tables 7, 8, and 9). This is the case for chemicals that produced thyroid tumors as well as for analogues that did not.

The genotoxic potential of 4,4'-oxydianiline was evaluated in more detail using information from the published literature (Appendix D) to supplement that generated by NTP (Table 3). It is concluded that it is a frame-shift and perhaps base-pair substitution mutagen in Salmonella that requires metabolic activation for an effect to be noted. In keeping with its mutagenic effects on bacteria, 4,4'-oxydianiline also produced gene mutations, chromosome aberrations, and sister chromatid exchanges (SCE) in cultured mammalian cells. However, SCE are not increased in vivo, and two DNA damage assays in vivo gave discordant results. In vitro transformation studies were generally positive. Thus, the analysis of 4,4'-oxydianiline confirms the suspicion from Tables 7 through 9 that aromatic amines are genotoxic agents.

(c) Complex halogenated hydrocarbons--For the class of halogenated hydrocarbons there are a few scattered positive genotoxicity results (3 out of 16 chemical-test comparisons among the agents producing thyroid tumors) (Table 10), although many compounds have not been well characterized as to gene mutations and chromosomal effects. Other than toxaphene, all compounds are negative in the Salmonella test. Structural analogues that have not produced thyroid tumors also show a paucity of genetic responses (7 positives among 17 comparisons). No firm conclusion can be drawn on these compounds because the data are limited but, in general, it appears that complex halogenated hydrocarbons fail to demonstrate much genotoxic potential.

(d) Amitrole--Amitrole has not been investigated by the NTP concerning its carcinogenicity, but from other long-term animal studies, it is known to produce

TABLE 7. GENOTOXICITY DATA FOR SINGLE RING AROMATIC AMINES

	GENE MUTATIONS			CHROMOSOMAL EFFECTS	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for Thyroid Tumors					
3-Amino-4-ethyloxyacetanilide	+/+	n	-	n	n
o-Anisidine hydrochloride	+	n	n	n	n
2,4-Diaminoanisole sulfate	+/+	+/+	n	u	u
HC Blue No. 1	+	+	-	+	+
2. Chemicals Negative for Thyroid Tumors					
p-Cresidine	+/+	n	n	n	n
5-Nitro-o-anisidine	+	n	?/-/?	n	n
p-Anisidine	-/+	n	n	w	+
2,4-Dimethyoxyaniline hydrochloride	+	+	n	+	+
m-Phenylenediamine	+	n	n	+	+
p-Phenylenediamine hydrochloride	+	+/+	u	+	+
2-Nitro-p-phenylenediamine	+	+/+	n	+	+

SYMBOLS: SA, Salmonella reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in Drosophila; CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; -, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

TABLE 8. GENOTOXICITY DATA FOR BRIDGED DOUBLE RING AROMATIC AMINES

	GENE MUTATIONS			CHROMOSOMAL EFFECTS	
	SA	ML	SLRL	CA	SCE
1. Chemicals Positive for Thyroid Tumors					
4,4'-Methylenedianiline dihydrochloride	+	+	n	+	+
4,4'-Methylenebis (N,N-dimethyl) benzenamine	-	+/+	n	n	n
4,4'-Thiodianiline	+	n	n	u	u
4,4'-Oxydianiline	+	+	n	+	+
2. Chemicals Negative for Thyroid Tumors					
Michler's ketone	+/+	+/+	n	-	-
4,4'-Sulfonyldianiline	-/-	-	n	+	+
Sulfisoxazole	-/-	+	u	-	+

SYMBOLS: SA, Salmonella reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in Drosophila; CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; -, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

TABLE 9. GENOTOXICITY DATA FOR MISCELLANEOUS AROMATIC AMINES

Chemicals Positive for Thyroid Tumors	GENE MUTATIONS			CHROMOSOMAL EFFECTS	
	SA	ML	SLRL	CA	SCE
C.I. Basic Red 9 monochloride	+/?	+/?	n	-	+
1,4-Naphthalenediamine	+	n	n	n	n

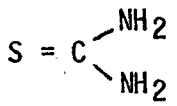
SYMBOLS: SA, Salmonella reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in Drosophila; CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; -, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

TABLE 10. GENOTOXICITY DATA FOR COMPLEX HALOGENATED HYDROCARBONS

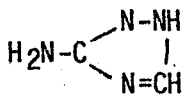
	GENE MUTATIONS			CHROMOSOMAL EFFECTS	
	SA	ML	SLRL	CA	SCE
1. Chemicals Positive for Thyroid Tumors					
Aldrin	s	n	n	n	n
Chlordane	(r) -	(t) +	n	(r) -	(r) +
Chlorinated paraffins (C_{12} 60% chlorine)	-	n	n	n	n
Decabromodiphenyl oxide	-	-	n	-	-
2,3,7,8-Tetrachlorodibenzo-p-dioxin	-	-	-	-	-
p,p'-Tetrachlorodiphenylethane (p,p'-DDD)	-	n	n	u	u
Toxaphene	+	n	n	n	n
2. Chemicals Negative for Thyroid Tumors					
Dieldrin	-	+	n	-	+
Heptachlor	-	u	n	+	+
Chlorinated paraffins (C_{23}, 43% chlorine)	-	n	n	n	n
PBB mixture (Firemaster FF-1)	-	-	n	-	-
p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE)	-	+	+/-	-	w

SYMBOLS: SA, *Salmonella* reverse mutation; ML, mouse lymphoma L5178Y cell thymidine kinase locus; SLRL, sex-linked recessive lethal in *Drosophila*; CA, chromosomal aberrations in CHO cells; SCE, sister chromatid exchange in CHO cells; s, selected for testing by NTP; r, reagent grade; t, technical grade; -, negative result; +, positive result; n, not tested; w, weak positive result; ?, equivocal result; /, results from two or more laboratories; u, under test by NTP.

thyroid, pituitary, and liver tumors (see Paynter et al., 1986). Like the thionamides and aromatic amines, amitrole inhibits thyroid peroxidase. Although it lacks the thiol group of thionamides, it does show some structural similarity (an $-N-\overset{R}{\underset{|}{C}}-N-$ grouping), as illustrated with the comparison with thiourea.



thiourea



amitrole

Gene mutation testing of amitrole has spanned prokaryotes, yeast, insects, and mammalian cells in culture (Appendix E). Many replications of bacterial testing in Salmonella and E. coli have almost uniformly failed to demonstrate mutagenic effects, which led a review group to declare amitrole negative (see Bridges et al., 1981). Point mutation tests in Saccharomyces and Drosophila were also negative (positive in one case; see Appendix E). Test results in mammalian cells in culture have been conflicting, with confirmed negative results in mouse lymphoma cells but positive effects in one laboratory for two different loci in Syrian hamster embryo cells. Thus, submammalian testing indicates little concern about point mutations, whereas results in mammalian cells are positive in Syrian hamster but not mouse cells.

Testing for chromosomal effects includes evaluation of numerical aberrations, structural aberrations, and sister chromatid exchange. Negative results have been obtained in yeast and insect nondisjunction systems and in mammalian cells in culture. Two in vivo mouse micronucleus assays, which can give some indication of numerical chromosome aberrations, were also negative.

Tests for structural chromosome aberrations have been uniformly negative and include the following: human lymphocytes in culture, mouse bone marrow

cytogenetics, and mouse micronucleus and dominant lethal tests.

An increase was reported in the frequency of SCE in CHO cells in culture in two studies; a negative response was recorded in a third study in the same cells.

DNA damage tests have been performed on bacteria, fungi, and mammalian cells in culture. Of six bacterial tests, five were reported as negative. Thus, there is little indication in bacteria of a DNA-interactive effect. Two of six DNA damage tests in Saccharomyces were positive. One such test in Aspergillus gave a weak positive reaction.

Increases in unscheduled DNA systhesis have been reported in human cells. For HeLa cells, a positive dose-response effect for amitrole was noted in the presence of rat liver S9; no such increase was noted in the absence of exogenous activation (Martin and McDermid, 1981). Also, amitrole was reported in an abstract to be positive in human EUE cells; the conditions of the study were not given.

Lastly, several positive studies have been reported for in vitro transformation in Syrian hamster and rat embryo cells, which argue for some type of genotoxic effect.

In sum, there is limited evidence for the genotoxicity of amitrole. This effect is probably not mediated through mutagenic mechanisms: there is no indication of the production of chromosomal mutations and, at best, the point mutagenic evidence is inconclusive. There are indications, however, that under some circumstances amitrole produces DNA-damaging effects. These results are augmented by confirmed positive responses in in vitro transformation. Thus, there is support for amitrole having a weak DNA-interactive or genotoxic effect that probably does not involve mutation per se.

(e) Conclusion--The review of three chemical classes demonstrating thyroid carcinogenesis illustrates that thyroid carcinogenesis is not uniformly tied to genotoxicity. Thionamides (and amitrole) and complex halogenated hydrocarbons demonstrated only limited indication of a genotoxic potential, whereas aromatic amines regularly showed positive short-term test results. Emphasis on this point is gained from review of structural analogues from these classes that did not produce thyroid tumors; their outcome on the tests was basically similar to that of the thyroid carcinogens. Thus, thyroid carcinogens do not show a consistent response on genotoxicity tests.

If we look at chemical classes as to their influence on thyroid peroxidase, we again fail to see a consistent pattern as to their genotoxicity. Chemicals from within the thionamides and aromatic amines (as well as amitrole) are known to inhibit thyroid peroxidase. However, the reviewed thionamides (and amitrole) are generally not genotoxic, whereas the amines are active. Thus, genotoxicity is not correlated with functional activity on peroxidase.

It is well recognized that aromatic amines are often carcinogenic in animals and that many means are available within organisms to activate these structures to reactive intermediates that have genotoxic potential. To the extent that certain aromatic amines also inhibit thyroid peroxidase, it seems possible that such agents may have two means to influence thyroid carcinogenesis: to induce DNA damage and to increase the output of TSH from the pituitary.

Although the remarks made in the previous paragraph are representative impressions of the data on chemical classes as a whole, they certainly do not necessarily apply to any one chemical within a class. Many times chemicals give a smattering of positive and negative results. In other cases, such as with the thionamides and amitrole, the evidence indicates a general lack of activity for some end points (e.g., gene mutations and chromosomal aberrations),

but the potential presence for other effects (e.g., *in vitro* transformation). Each of these cases makes it difficult to reach an all-inclusive position on genotoxicity. Still, within the limits of the present review, there does not seem to be a consistent relationship across chemical classes as to their ability to produce genotoxic effects.

VI. HUMAN DATA ON THYROID HYPERPLASIA AND NEOPLASIA

The goal of this section is to compare human and animal information bearing on thyroid physiology, disruption of thyroid function and development of hyperplasia (goiter) and neoplasia. As has been related, it has been well established by long-term experiments in animals that certain chemical substances and other treatments cause thyroid hyperplasia that will progress to neoplasia. While evaluation of laboratory experiments garners useful information on likely processes in humans, verification of this for human thyroid carcinogenesis requires evaluating the weight of evidence from several different approaches and merging data from clinical observations, studies of clinical populations, and epidemiologic studies.

Currently, the only verified cause of thyroid cancer in humans is x-irradiation (Ron and Modan, 1982; NCRP, 1985), and this finding is well documented in experimental animals. There are conflicting data in humans bearing on an association of iodine deficiency and thyroid cancer, unlike the case in animals where the association is well established. In contrast to the situation in animal studies, no studies follow a single human population directly through the sequence from exposure to chemical substances or initiation of some other treatment through hyperplasia and eventually to neoplasia. Consequently, the information on humans must be analyzed in separate steps, describing the role of certain treatments on the development of hyperplasia and then describing risk factors or antecedent conditions for thyroid neoplasia. The combination of these two analyses allows one to make some inferences about the overall comparability of animal models and humans regarding thyroid carcinogenesis.

A. THYROID-PITUITARY FUNCTION

It is widely accepted that the pituitary-thyroid axis and the nature, body handling, and function of thyroid hormones and TSH are quite similar in experimental animals and humans. For instance, in a review of thyroid function in humans, Larsen (1982) presented clinical data on the feedback regulation of thyrotropin secretion by thyroid hormones and the tissue conversion of T_4 to T_3 that is basically like that in experimental animals. Recent evidence, however, helps to point out some of the differences that may exist between animals and humans. For instance, in the rat there is active conversion of T_4 to T_3 which then regulates TSH production, whereas in humans circulating T_3 may play a more dominant role (Fish et al., 1987).

B. CAUSES OF THYROID HYPERPLASIA

Animals and humans respond similarly to a number of treatments that disrupt thyroid function such as 1) a lack of dietary iodide, 2) blockage of the iodide transport mechanism (ionic inhibitors), 3) interference with the synthesis of thyroid hormone (peroxidase inhibition), 4) suppression of thyroid activity by high concentrations of iodide, 5) enhanced peripheral metabolism of thyroid hormones, and 6) damage to the thyroid gland by ionizing radiation (see Sections III and V.C. of this report; Gilman and Murad, 1975; Green, 1978; Paynter et al., 1986; De Groot and Stanbury, 1975; Meyers et al., 1976). Each of these can lead to goiters in humans.

1. Chemical Inhibitors

Several examples of chemical substances that influence thyroid status in humans are summarized in Table 11 to illustrate the nature of the effects. The agents include such things as thyroid peroxidase inhibitors (e.g., ethylene thiourea, sulphonylureas, resorcinol), a cation (lithium), an organiodide (amiodarone), and inducers of mixed function oxidases (phenobarbital, PBB). In

TABLE 11. STUDIES ON HUMANS INDICATING EFFECTS OF CHEMICALS ON THYROID-PITUITARY FUNCTIONS

Chemical	n=	Dose or Exposure[a]	Health Status[b]	Effects[c,d]	Temporal[e]	Data Base[f]	Ref.
Amiodarone	229 treated ≥83 cardiac	~270 mg/day >17 months	Chronic treatment for cardiac disorders, male and female; from Italy and Massachusetts. Cardiac and normal controls. 161 treated were euthyroid.	Italy: 10% hyperthyroidism 5% hypothyroidism; Massachusetts: 2% hyperthyroidism, 22% hypothyroidism.	Response blunted with chronic treatment.	Other studies concur that hyperthyroidism is seen less in areas of sufficient I^- intake. Relationship well characterized.	Martino et al., 1984
Carbamazepine (CBZ)	27 83 controls	Dose not stated, CBZ alone or with phenobarbitone. Long-term.	Adult epilepsy patients, long-term therapy. Controls euthyroid.	↓T_4*, no change in T_3, no change in TSH.		One other report.	Rootwelt et al., 1978
	7	CBZ alone.	New patients, basal and treatment values.	↓T_3 and FT_3 index*; ↑FT_4 index↓*; ↑TSH* to day 20 then ↓.	Perhaps delayed effect due to enzyme.		
Ethionamide	2	1 g/day plus other drugs	48-year-old female with TB; 54-year-old male with TB and diabetes.	↓T_4, ↑TSH, goiter in female	Symptoms and T_4 normal after drug removal.	One other report had unclear etiology.	Moulding and Fraser, 1970
Ethylenethiourea	46 40 controls	In air 10-240 ug/m^3	Male workers and controls with no history of thyroid disease.	↓T_4*; normal TSH. 1-hypothyroidism.	Lower T_4 in more exposed group.	Three studies in workers, no or slight changes in thyroid function.	Smith, 1984

Table 11 contiued on next page

TABLE 11. (continued)

Chemical	n=	Dose or Exposure[a]	Health Status[b]	Effects[c,d]	Temporal[e]	Data Base[f]	Ref.
Lithium	86 105 controls	Male, 32 (18-48) mEq/day; female, 26.7 (8-48) mEq/day; 3-169 months.	Manic-depressive. Outpatient male and female.	↓T_3;↓T_4; ↑TSH in females; hypothyroidism.		Several studies report high frequency of hypothyroidism especially in women; goiter reported only in females. TSH considered diagnosed.	Transbol et al., 1978
Oxyphenbutazone	1	Not stated	63-year old female with back pain.	↓T_3;↑ I uptake ↑ hypothyroidism.	Remission after drug removal.	First report; phenylbutazone known to be goitrogen.	Lane et al., 1977
Phenytoin	10 83 controls	Not stated	Adult epilepsy patients on long-term treatment.	↓ no change in T_3;↓T_4*; no change in TSH.	Similar to carbamazepine therapy.	Literature agrees on ↓ T_4.	Rootwelt et al., 1978
Polybrominated Biphenyls (PBB)	35 89 controls	Occupational exposure;>6 weeks, polybrominated biphenyl oxide	Male free of thyroid disease.	Low T_4; ↑TSH; no goiter; 4/35 hypothyroidism. Antithyroid antibodies in some.	↓ T_4 may persist after exposure ceases.	No other reports. Thyroid abnormalities in rats given PPBs and polybrominates biphenyl oxide.	Bahn et al., 1980
Sulphonylureas	220	42-60 months treatment, average duration. 0.6-3.0 g/day tolbutamide or 0.1-0.5 g/day chloro-	Diabetics with no ↑ blood urea. Groups age and sex matched.	↓ serum PBI, ↑ incidence with duration of treatment; 0 goiter; ↑ hypothyroidism.	PBI to normal after treatment stopped and drops again when treatment resumed.	Several other studies of ↓ PBI, ↓ in ^{131}I uptake; no hypothyroidism with short-term treatment. Carbutamide - more pronounced effects.	Hunton et al., 1965

Table 11 continued on next page

TABLE 11. (continued)

Chemical	n=	Dose or Exposure[a]	Health Status[b]	Effects[c,d]	Temporal[e]	Data Base[f]	Ref.
Sulphonyl-ureas (continued)	229 controls	Diet alone, 113; insulin, 93; bilguan-ides, 23.	Diabetics				
Resorcinol	3	Ointment on leg ulcers.	Females, 50, 59, 60 years. 1-cardiac, all clinical hypothyroidism cases.	↓ PBI in 2; rapid development of severe symptoms.	↑ ^{131}I uptake when treat-ment stopped and hypo-thyroidism reversed.	A 1977 report of hypothyroid-ism in a dialy-sis patient cites only this reference.	Bull and Fraser, 1950

[a] In some cases exposures include other drugs or chemicals. Only the dose of the suspected goitrogen is given. To show the association of that chemical with thyroid dysfunction look for remission after removal (Column 6).

[b] Subjects assessed as euthyroid prior to treatment or exposure is so stated.

[c] Effects examined varied among studies. The column reports results of five items; serum T_3, T_4 and TSH; thyroid gland enlargement; clinical thyroid dysfunction. Other items examined are not reported in the table. Absence of entry indicates effect not assessed.

[d] Symbols: * Statistically significant at ≤ 0.05. Specified only if test used is stated in text and appropriate. However, testing may vary among studies e.g., most are tests of mean differences, but Bahn et al. (1980) test differences in number with elevated levels between groups.

↑ = increase

↓ = decrease

T_3 and T_4 = serum levels

hypo- and hyperthyroidism refer to clinical observation

[e] Time-related effects seen as a result of repeated tests, withdrawal of treatment, or resuming treatment.

[f] Existing data base to support goitrogenic potential of chemical as reflected in this reference.

each case exposures result in reduction in circulating thyroid hormone levels and in some cases elevated TSH levels or goiters. These responses are like those seen in animals.

Because the data base varies among the chemicals, a summary of supporting references, including those reported in the study, is included in a separate column entitled "data base." For example, the goitrogenic effect in humans of sulfonylureas and of amiodarone has been reported in several clinical studies. Differences in quantitative value of the results among studies are to be expected because of differences in health status, age, sex, and dietary factors. In some studies these factors are controlled (patients of similar age) or evaluated in the analysis (sex differences).

The value of a case report in support of the hypothesis is strengthened if cessation of treatment with the putative goitrogen or other agent is followed by a return of thyroid function tests to normal. These temporal associations are important in assessing the evidence for the association because subjects are exposed to other drugs or possible confounding factors. This information, which is important in assessing the strength of the evidence, is summarized in the table column titled "Temporal." Prospective clinical studies provide valuable information because subjects are euthyroid prior to exposure.

Other observations point out the comparability of response in humans as in animals. In hypothyroid animals the cells of the pituitary enlarge and become "thyroidectomy cells" (Baker and Yu, 1921) and, according to some authors, may undergo hyperplasia and finally neoplasia (see Section IV.B.). Indirect studies in humans also demonstrate some of these findings. The bony covering of the human pituitary, the sella turcica, normally enlarges with age up to about 20 years and then remains essentially constant in size. Enlargement in the sella turcica beyond normal limits is noted in cases of hypothyroidism,

and there is an inverse relationship between the blood levels of thyroid hormones and sella size and a direct one between TSH levels and size of the sella turcica (Yamada et al., 1976; Bigos et al., 1978). It is interesting to note that there are also a few clinical reports linking chemical hypothyroidism and pituitary adenomas, and at least some of them appear to be TSH-secreting tumors (e.g., Samaan et al., 1977; Katz et al., 1980; see review by Balsam and Oppenheimer, 1975), although the case is not established with any certainty.

2. Dietary Factors

Much of the human investigations of disruption in thyroid function following environmental modifications have come from the study of populations where there are dietary changes, namely deficiency of iodide and the consumption of foods containing goitrogenic substances.

a. Iodine Deficiency--The most striking patterns of the geographic distribution of populations with goiter is attributed to deficiency of iodine in the diet as a result of low environmental iodine levels. Endemic goiter has occurred throughout the world, particularly in mountainous areas such as the Alps, Himalayas, and Andes, and in the United States in areas around the Great Lakes. De Groot and Stanbury (1975) cite the report of thyroid hyperplasia in domestic goats and in wild rodents in endemic areas of iodine deficiency in the Himalayas, which again points out the similarity of response among mammals. Goiter incidence has been virtually eliminated in the United States and Europe by the introduction of iodized salt (Williams, 1977; De Groot and Stanbury, 1975; Hedinger, 1981).

Several arguments support iodine deficiency as a cause of goiter: 1) there is an inverse correlation between iodine content of soil and water and the appearance of goiter in the population; 2) metabolism of iodine and TH and TSH status in patients with this disorder fits the pattern expected and is reversed with iodine prophylaxis; and 3) there is a sharp reduction in goiter prevalence with iodine prophylaxis (Williams, 1977; Hedinger, 1981).

Iodine deficiency in humans can result in profound thyroid hyperplasia. Goiters up to 5 kg (a 100-fold increase in weight) g have been observed in iodine-deficient areas as a compensatory response to inability to synthesize thyroid hormone. Generally, the impairment in hormone synthesis is overcome in time, and the individual becomes clinically euthyroid, even in the presence of some derangement in T_4 and TSH levels. Often in goitrous populations repeated cycles of hyperplasia and involution occur which can lead to multinodular goiter. In contrast to the hyperplastic goiter, multinodular goiters do not regress upon administration of iodine. Likewise, thyroid hormone usually has no effect on long-standing goiters (Ingbar and Woeber, 1981). Adenomatous hyperplasia is a less common cause of nodularity but is significant, because it is difficult to distinguish from neoplasia, thus complicating the assessment of the association between hyperplasia and neoplasia. As will be developed later in this section, it does not appear that thyroid cancer is a major problem arising from iodine-deficient goiters, in contrast to the observations in experimental animals which indicate that tumors frequently arise under iodine-deficient conditions.

b. Other Goitrogens--Observations of goiter distribution suggest that factors other than iodine deficiency could be important. The incidence of goiter varies within the population in endemic areas, and the severity is not uniform among all inhabitants; these suggest the presence of risk factors in addition to iodine deficiency. Although it is considered unlikely that natural goitrogens in food are a primary cause of goiter in humans, variability in response within endemic areas has led some to conclude (De Groot and Stanbury, 1975) that "natural goitrogens acting in concert with iodine deficiency may determine the pattern and severity of goiter."

As discussed in Section V.B. a thionamide, goitrin, with antithyroid

activity in animals and in humans, has been isolated from certain cruciferous foods (e.g., turnips). It exists naturally as progoitrin, an inactive thioglycoside, which is hydrolyzed in vivo to goitrin.

Human data exists to illustrate the thyroid inhibiting effect of the monovalent hydrated anion, thiocyanate (TCN), and of cyanogenic glucosides, that are hydrolyzed in the body to thiocyanate. TCN blocks the uptake of iodide into the thyroid. Chemicals that are metabolized to thiocyanates are found in seeds of the plants of the genus Brassica, in Cruciferae, Compositae and Umbelliferae. These include cabbage, kale, brussel sprouts, cauliflower, turnips, rutabagas, mustard, and horseradish. The effect was established in man as a result of clinical use of potassium thiocyanate (Gilman and Murad, 1975).

It has been assumed, therefore, that eating foods producing the thiocyanate ion or goitrin contributes to endemic goiter. De Groot and Stanbury (1975) cite studies in Australia, Finland, and England, that suggest cattle have passed these goitrogens to humans through milk. Progoitrin has been detected in commercial milk in goitrous regions of Finland, but not in nongoitrous regions. Seasonal development of goiter in school children has heen related to milk from cows fed kale (De Groot and Stanbury, 1975).

Several dietary items that are staples in some cultures contain cyanogenic glucosides. These include cassava, sorghum, maize, and millet. In its raw form, cassava contains toxic levels of cyanogenic glucoside, and although much of it is removed by pounding and soaking, poorly detoxified cassava is a suspected cause of goiter in Central Africa.

Recent studies in Africa contribute more direct evidence to support an interactive effect of TCN (or cyanogenic glucosides) and a diet low in iodine. In an iodine-deficient region of the Sudan where goiter prevalence may reach 55%, the frequency of large goiters is higher in rural than in urban areas

(Eltom et al., 1985). The predominant staple food in rural Darfur is millet. Rural subjects with goiters had statistically significantly higher levels of TSH and T_3, lower levels of T_4 and free T_4 index than urban subjects with goiters. Serum TCN was significantly higher in rural subjects, but the elevated levels of urinary TCN did not reach statistical significance. The urinary iodine excretion, a reflection of quantity of iodine ingested, was not significantly different between the two groups. These results are consistent with the hypothesis that TCN overload in conjunction with iodine-deficiency causes more severe thyroid dysfunction than iodine-deficiency alone. Evidence of a possible effect has also been reported in North Zaire in Central Africa in children with iodine-deficiency (Vanderpas et al., 1984).

C. CAUSES OF THYROID CANCER IN HUMANS

Epidemiologists search for clues to causes of disease and to factors that increase an individual's risk of disease (risk factors) by examining descriptive data or designing analytic studies. Descriptive data consist of morbidity, mortality, or incidence rates of diseases in population groups. Incidence rates (newly diagnosed cases in a population over a given time period) reveal patterns of disease by age, race, sex, ethnic group, and geographic locale. These rates and their changes over time and space identify high risk groups and provide indirect evidence for causes of disease. Associations between host factors and disease are hypothesized.

Analytical epidemiology consists of case-control, often termed retrospective, and cohort or prospective studies. These studies permit greater control of confounding factors and opportunity to link exposure and response information in individuals. Thus, evidence for causes of disease is more direct.

As a result of descriptive and analytic epidemiologic data, radiation is a well documented cause of thyroid cancer in humans (Schottenfeld and Gershman, 1978;

Ron and Modan, 1982). Incidence rates for thyroid cancer rose roughly two-fold between the 1940s and the 1970s for persons under age 55. The change in pattern coincides with administration of x-ray for various medical treatments and is consistent with the hypothesis that ionizing radiation is a cause of thyroid cancer in children and young adults. Childhood irradiation was observed more often in thyroid cancer cases than controls. Ron and Modan (1982) summarize eight epidemiologic studies of populations exposed to x-ray therapy, atomic-bomb explosions, and fallout from nuclear weapons testing.

The epidemiologic approach to investigating whether hyperplasia (goiter) leads to thyroid cancer in humans is to: 1) examine descriptive data, 2) compare the cancer rates between endemic goiter areas and goiter-free areas, 3) examine time trends for thyroid cancer after prophylactic measures (iodine supplementation) reduce endemic goiter frequency in a given area, and 4) evaluate whether goitrous individuals have a greater risk of thyroid cancer or whether thyroid cancer cases have a more frequent history of hyperplasia and nodules than controls. These steps are summarized in the sections below.

1. Descriptive Epidemiology

Variations in cancer incidence rates by country and race may be studied to evaluate the role of host and environmental factors on disease. Despite the striking geographic patterns for goiter, no similar trends are detected for incidence of thyroid carcinomas in the areas for which cancer incidence data are available. It is one of the rarest and generally least virulent carcinomas, and although it has increased somewhat in recent decades, purportedly because of medical radiation exposure, it is not considered a major public health problem (Ron and Modan, 1982).

For several countries, thyroid cancer shows rising age-adjusted incidence rates with age and consistently higher rates for women than men, particularly

in young adults. Rates for males range from 0.6 to 5 per 100,000 and for females from 1.2 to 16 per 100,000. Variations by country are relatively small compared with that for other cancer sites (about 10-fold) and are not consistently related to geography or race. The highest age-adjusted rates in females (1967-1971) were for Hawaiians in Hawaii (16/100,000), Iceland (16.3/100,000), and Israeli Jews (8.3/100,000) (Waterhouse et al., 1976).

The incidence of thyroid cancer detected clinically shows interesting distinctions from prevalence of occult thyroid cancer detected at autopsy. At autopsy, thyroid carcinoma is equally frequent in men and women, and high rates have been diagnosed in populations that have unremarkable clinical rates of thyroid cancer (Shottenfeld and Gershman, 1978). These observations have led these authors and others to hypothesize that the host and environmental factors that enhance the development of clinically detected thyroid cancer are different from those that incite tumorigenesis.

Experimental evidence in several laboratory species demonstrates that iodine deficiency, certain chemicals, and other causes of prolonged TSH stimulation result in thyroid enlargements and eventually thyroid tumors. In the absence of such information in humans other studies need to be conducted to get some handle on human thyroid carcinogenesis.

Much of the work on the relationship between goiter and thyroid cancer has focused on populations differing in iodine intake, since iodine-deficiency (endemic goiter) has been and still remains a major health problem in various parts of the world. Numerous reviews of the subject have been written which conclude that past studies are conflicting about the role of goiter in thyroid carcinogenesis (e.g., Alderson, 1980; Hedinger, 1981; Riccabona, 1982). Doniach (1970a) reviews much of the information available to that time and questions the link between endemic goiter and thyroid cancer development.

In geographical epidemiologic studies, thyroid cancer rates are compared in geographical areas with different goiter rates. Wegelin (1928) compared the frequency of thyroid cancer in an autopsy series in five areas. The largest percentage with thyroid cancer occurred in Berne, Switzerland, an area where goiter was highly endemic. The lowest percentage of cancer appeared in Berlin where endemic goiter was rare. Other geographic correlation studies have followed, yet reports have been conflicting. For example, no correlations were found in reports from Australia and Finland (Alderson, 1980; Ron and Modan, 1982), and Pendergrast (1961) found no associated increase in the cancer rates in goiter areas in the United States compared with non-goiter areas. Hedinger (1981) cites incidence statistics that show no decline in frequency of thyroid malignancies despite the virtual elimination of goiter by iodine prophylaxis. On the other hand, Wahner et al. (1966) did show a positive correlation when they compared the incidence of thyroid cancer in Cali, Colombia, an endemic goiter area, to similar data in New York State and Puerto Rico. Thyroid cancer rates for both sexes were about three times higher in Colombia than in the other two sites.

Several reasons may account for differing study outcomes. Some of the correlations are based on reports of high thyroid cancer rates generated from pathology studies of surgery cases, and are likely to suffer from a selection bias because thyroid disease suspected of carcinogenicity is likely to be referred to surgery (De Groot, 1975). Different causes of cancer may result in different histopathological types of thyroid cancer. In the United States, in particular, radiation-induced cancer associated with therapy in childhood could have masked a decrease associated with iodine prophylaxis. After the introduction of iodized table salt in Switzerland and decreasing incidence of goiter, thyroid cancer rates remained stable but an increasing proportion of thyroid cancers

were classified as papillary (Shottenfeld and Gershman, 1977). Therefore, the conflicting data cited above are inconclusive and difficult to interpret.

More recent geographical studies consider the histological type of thyroid cancer. In Cali, Colombia, an endemic goiter area, at least 90% of the follicular and anaplastic cancer specimens showed evidence of goiter, whereas about 50% of the papillary tumors were associated with goiter (Wahner et al., 1966). These results suggest some relationship between goiter and the histological type of cancer.

In Zurich, Switzerland before the advent of iodine supplementation, few of the tumors were papillary (7.8%), whereas after that time the proportion of papillary cancers among the total increased (33.4%) while the proportion of follicular and anaplastic tumors decreased (Hedinger, 1981; Riccabona, 1982). Since papillary cancers have the best prognosis and anaplastic the worst, with follicular intermediate, these results suggest that thyroid cancer in endemic goiter regions may be associated with more aggressive forms of cancer.

Further evidence of a relationship between iodine intake (from inadequate to hypernormal) and the form of thyroid cancer comes from a review of thyroid cancer cases coming to surgery in Northeast Scotland, a region with average iodide intake, and Iceland, an island with very high iodide intake (Williams et al., 1977). Persons from Iceland have unusually small thyroid glands, high concentrations of iodide in plasma and the thyroid gland, and low plasma TSH levels. Papillary cancer incidence was about five fold higher and the proportion of papillary cancers among the total was greater in Iceland than in Scotland (71% vs. 54%). Offsetting the difference in papillary cancers, the proportion of follicular tumors was comparable in the two groups, but anaplastic cancers were more common in Scotland than Iceland (19% vs. 10%).

In contrast to the above studies suggesting some relationship between

iodide intake and the form of thyroid cancer in humans, others fail to support this hypothesis. For instance, Waterhouse et al. (1982) report that the relative frequencies of the major histological types for several countries show the highest proportion of follicular carcinoma in Sao Paulo, Brazil, Bombay, India, and Zaragoza, Spain--all areas not noted for endemic goiter. The highest proportion of papillary carcinoma was reported from all North America cancer registries, and from Hawaii, Israel, and Singapore. In addition to noting the potential for disagreement in diagnoses among experienced pathologists, the authors conclude that the significance of these differences is unclear. Therefore, geograghic correlations with and without histology data are inconclusive and do not show a consistent relationship between endemic goiter areas and thyroid cancer rates.

Probably the most profound disruptions in thyroid functioning occur in cases of familial goiter where there are inherited blocks in thyroid hormone production (Stanbury et al., 1979). When left untreated, these patients develop profound hyperplasia and nodular (benign tumor) changes, but only a very few cases have gone on to develop thyroid carcinoma (see review by Vickery, 1981). Like with endemic goiter, it appears that the hyperplastic thyroids in these patients do not often undergo malignant transformation; this contrasts with the findings in long-term animal studies where blocks in thyroid production regularly lead to thyroid cancer.

Although not much seems to have been done concerning the follow-up of patients with Graves' disease (hyperthyroidism) as to thyroid cancer development, the little that has been done (a follow-up of 30,000 patients) suggests there may not be a significant thyroid cancer problem in these cases (Dobyns et al., 1974; see also Doniach, 1970a). [One very small study of Graves' patients suggested a higher than expected frequency of thyroid cancer (Shapiro et al., 1970)].

The reason Graves' patients may be at risk is the finding that many of the persons carry immunoglobulins in their blood which bind to the TSH receptor on thyroid cells and, at last *in vitro*, act like TSH to stimulate DNA synthesis and cell division (Valente et al., 1983; Tramontano et al., 1986b). Since these patients frequently have enlarged thyroid glands, one can not help but think that the immunoglobulins may stimulate thyroid cell division *in vivo* as well.

The single investigation of Graves' disease patients treated with antithyroid agents (i.e., thionamides) for at least one year failed to show any thyroid cancers in over 1,000 patients (Dobyns et al., 1974). Again, this suggests that at least circumscribed use of antithyroid drugs is not attended with a marked thyroid cancer risk. It should be pointed out, however, that the goal of antithyroid treatment for Graves' disease is to bring patients into euthyroid and not a hypothyroid status where increases in TSH may occur. Thus, the follow-up of treated cases of Graves' disease does not provide significant evidence to impugn or acquit antithyroid agents.

2. Analytical Epidemiology

Of all the various types of data on humans from which causal associations can be inferred, the strongest evidence is derived from analytical epidemiology --cohort or case-control studies--that evaluate data on individuals and suitable controls. Analytical epidemiologic studies have helped to establish ionizing radiation as a cause of thyroid cancer (Ron and Modan, 1982).

Three case-control studies of thyroid carcinoma in the United States have recently been completed which evaluated risk factors for cancer including pre-existing thyroid disease (Table 12). These studies were designed to test a potential hypothesized role of endogenous female hormones in thyroid cancer. Hormonal factors are suspected as a cause of thyroid cancer because of the

TABLE 12. EPIDEMIOLOGIC STUDIES OF THYROID CANCER AND ITS RELATIONSHIP TO GOITER AND THYROID NODULES

Odds Ratio (95% confidence limits)[a]		Comment	Reference
Goiter	Thyroid Nodules		
4.5 (1.6-12.2)[b]	8 7 (1.6-47.5)[b]	Women aged 18-80	McTiernan et al., 1984
10.5 (2.5-44.8)[c]		White women aged 15-40	Preston-Martin et al., 1987
5.6 (1.0-41)[d]	33 (4.5-691)[d]	Adjusted for age, sex and prior radiation exposure	Ron et al., 1987

[a]Odds ratio estimates risk of disease with the trait (or exposure) compared to risk without the trait. Confidence limits that overlap 1.0 are not significant.

[b]Data for those unexposed to radiation. The risk for all cases was goiter 6.6 (2.8-15.6) and nodules 12.0 (2.3-63.8).

[c]Presence of goiter or benign nodules.

[d]These data are from univariate analysis. The odds ratio of a multiple logistic regression adjusted for age and sex were thyroid nodules (28.0) and goiter (3.8) (not significant).

consistently higher rates in females and the peak occurrence in females at between ages 15 and 29 when hormonal activity is enhanced (Henderson et al., 1982; Ron and Modan, 1982).

McTiernan et al. (1984) studied 183 women aged 18 to 80 located from a population-based cancer surveillance system and 394 controls. The two groups had similar family history, weight, and smoking habits. The most common confounding factor in the analyses was age; therefore, relationships were adjusted to five age groups.

History of goiter for individuals unexposed to radiation showed a statistically significant and high odds ratio (OR) equal to 4.5. Further analysis of pre-existing goiter by histopathological type resulted in an OR=16.4 for follicular compared with 3.3 for papillary cancer. Radiation exposure doubled the risk for those with papillary histology, but did not change the risk for follicular. Thyroid nodules were also a statistically significant risk factor in those unexposed to radiation (OR=8.7) and was strongly related to papillary or mixed papillary-follicular thyroid cancer.

There are some potential biases in the McTiernan et al.(1984) study such as recall bias, relatively low ascertainment rate (65%), the lack of re-evaulation of the histopathology, and the reliance on telephone interviews rather than medical history. However, it is doubtful that these could be the cause of associations of the magnitude noted.

Preston-Martin et al. (1986) conducted a case-control study in which they questioned 110 female cases aged 15 to 40 and an equal number of matched controls. Diagnoses of cases were histologically confirmed, and thyroid disease was recorded if a physician was consulted at least 2 years prior to the cancer diagnosis. Statistically significant risk factors were found for thyroid enlargement as an adolescent (OR=10) and any goiter or benign nodules (OR=10.5).

The odds ratio of any thyroid disease was 14.5. The small number of cases of follicular carcinoma prevented analysis by histological type.

Ron et al. (1987) also found increased risk with parity as well as increased risk with goiter and nodules. This case-control study included 159 cases (109 female and 59 male) ascertained through a cancer registry and 318 controls from the general population. A review of the pathology was included. Thyroid nodules were evaluated separately from goiter and had a far greater risk (OR=33) compared with goiter (OR=5.6); both were statistically significant. The authors offer as caveats the fact that thyroid disease status was not medically verified and the response rate was only 62%.

In conclusion, these three recent case-control studies in the United States consistently showed thyroid cancer strongly related to pre-existing goiter and to thyroid nodules (Table 12). There is insufficient evidence to identify a quantitative difference in this relationship between follicular or papillary tumor types. One concern is that the associations between thyroid disease and thyroid cancer may be increased as a result of closer medical attention; after all, there must have been some clinical indication that the patients may have had a thyroid neoplasm prior to the time of surgery (like the presence of a nodule in the gland). However, the consistency among studies, the strength of the association, and the consistency with established causes (e.g., in all studies, ORs were increased with radiation) strongly support the hypothesis that thyroid nodules and, to a lesser degree, goiter are risk factors (potential causes) of thyroid cancer in humans. It should be pointed out, however, that in the two studies that analyzed for an association between hypothyroidism and thyroid cancer, neither showed a relationship (McTiernan et al., 1984; Ron et al., 1987).

VII. DEVELOPMENT OF SCIENCE POLICY

This section assembles pertinent points from the preceding review into a rationale for a science policy. It then lays out a set of principles that will help guide EPA in performing risk assessments on chemicals that have been shown or may have the potential to produce thyroid follicular cell tumors.

A. RATIONALE FOR SCIENCE POLICY

Carcinogenesis is considered to be a multistage process in which a number of endogenous and/or exogenous factors combine, either simultaneously or in sequence, to disrupt normal cell growth and function. Consequently, chemical carcinogenicity should not be viewed as a unique property of a chemical, but rather as an outcome of the interaction of a chemical with a complex biological system. A corollary to this is that cancer is a multifactorial disease that may occur through a number of different mechanisms.

The development of cancer has often been divided into three major stages: initiation, promotion, and progression. Initiation refers to the process whereby a chemical or other agent permanently alters the DNA of the cell. Promotion describes the subsequent processes involving the proliferation of the "transformed" cell through several steps (e.g., hyperplasia, neoplasia) leading eventually to a malignant tumor, while progression refers to the development of aggressive cell behavior including local invasion and distant metastasis. It is now recognized that initiation, promotion, and progression may each consist of several stages involving different mechanisms. It is believed that some of these stages are reversible and some are not; most appear to be susceptible to modulation (enhancement or inhibition) by a variety of exogenous (e.g., diet, stress, chemicals) or endogenous (e.g., age, sex, hormonal balance, health status) factors.

For most chemical substances one usually has too little relevant biological information on mechanism of action to be able to evaluate if or how that agent may be influencing the various stages of carcinogenesis. In some cases the results of genotoxicity testing may give clues as to the potential to initiate carcinogenesis, since initiation is thought to involve alterations in the DNA. However, chemicals that can initiate carcinogenesis can very often also complete the remaining stages in the carcinogenic process and lead to tumors.

Traditionally within EPA, chemicals that produce carcinogenic effects have been assessed as if they are "complete" carcinogens with both initiation and promotion components. Using this position as a basis, the Agency has generally assumed that any exposure to the chemical substance is attended with some small but finite risk of cancer. In modeling such dose-response relationships, an extrapolation procedure which has a low-dose linear function has been employed to estimate an upper bound on the additional lifetime cancer risks.

The 1986 EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) require the selection of a dose-response extrapolation model for each carcinogenic agent under review. A similar directive, with guidance to aid in the selection, is given to all federal agencies in the Office of Science and Technology Policy Cancer Principles (OSTP, 1985). The EPA Guidelines say that the Agency "will review each assessment as to the evidence on carcinogenesis [sic] mechanisms and other biological and statistical evidence that indicates the suitability of a particular model." In the case of certain kinds of thyroid carcinogenesis, there is considerable mechanistic information which can be used in making judgments about model selection. The remainder of this section will be devoted to laying out a rationale for assessing thyroid follicular cell carcinogenesis.

To fulfill their many critical functional roles, thyroid hormone levels in the circulation are maintained under strict homeostatic control. Homeostasis

is maintained primarily by a physiological feedback mechanism involving the controlled synthesis and release of thyroid stimulating hormone (TSH) from the pituitary in amounts that reflect the body's need for additional thyroid hormones. Consequently, the thyroid and the pituitary continually respond to both internal (physiological) and external (environmental) stimuli that increase or decrease the body's need for thyroid hormones. Failure to maintain homeostasis may result in sustained increases or decreases in circulating levels of thyroid hormones leading to hyperthyroidism and hypothyroidism, respectively.

Experimental studies in laboratory animals show that thyroid hyperplasia and neoplasia are most often associated with prolonged exposure to excessively high levels of TSH, irrespective of whether the latter results from endogenous or exogenous stimuli. Thus, thyroid neoplasia may arise as a result of chronic iodine deficiency, subtotal thyroidectomy, or the transplantation of hormonally active pituitary tumors, all of which are associated with long-term elevated TSH levels. Further evidence for the central role of TSH in the neoplastic process is the finding that treatments that lower circulating levels of TSH (e.g., hypophysectomy, thyroid hormone administration) prevent the development of hyperplasia and neoplasia or cause the reversal of hyperplasia towards a normal histological state.

Precise details of the mechanism through which prolonged elevated levels of TSH may lead to thyroid neoplasia remain to be elucidated. Recent research in molecular biology indicates that the induction of cell division (which can lead to hyperplasia) and the change from normal to transformed (neoplastic) cells are very complex processes. However, for certain thyroid tumors, some of the steps seem to include the following. TSH interacts with thyroid cells via specific plasma membrane receptors which leads to the induction of adenyl cyclase and cellular protooncogenes (c-fos and c-myc). It appears that TSH-

stimulated effects, in conjunction with the effects of other factors (e.g., somatomedins, epidermal growth factor, and phosphoinositol-mediated processes involving protein kinase c), commit the thyroid cell to DNA synthesis and cell division. Probably other interactions between TSH and other factors and influences (e.g., mutation, oncogene activation) enhance cellular transformation. Thyroid neoplasia, therefore, probably results from prolonged TSH stimulation in concert with other cellular processes.

Thus, it would seem that experimental procedures (like subtotal thyroidectomy) which stimulate increased levels of TSH may be influencing thyroid cells in at least two different but not necessarily independent ways. First, TSH provides a strong stimulus for cell division and the development of hyperplasia (oncogene expression probably plays a role here). However, it seems that TSH has finite ability to stimulate thyroid cell division both in vivo and in vitro. Thus, for thyroid cells to keep dividing as part of the carcinogenic process, it would appear that they are responding to factors in addition to TSH, or the cells themselves become changed. Second, TSH actions (like protooncogene induction) in concert with other cellular processes lead (by some yet undiscovered means) to neoplastic transformation.

Fitting the available information on thyroid follicular cell carcinogensis into the "traditional" three-stage model of carcinogenesis--initiation, promotion, and progression--is not easy (OSTP, 1985; Nowell, 1986). Although the effect of TSH (and other factors) on cell division is consistent with the concept of promotion, a hypothesis for the way TSH might "initiate" the carcinogenic process or enhance progression of neoplastic cells toward more malignant expression (local invasion and distant metastasis) is less straightforward. Since little is known about progression in thyroid carcinogenesis, remarks will be limited to initiation. At this time it appears that oncogene expression is dependent

upon the continued presence of TSH working via cyclic AMP. When TSH is removed, the stimulus for oncogene expression probably ceases. Given that transformation appears to require factors in addition to TSH, it is possible that the other factors complete the transformation process (i.e., initiation) begun by TSH. Another possibility would be that "spontaneous" events like mutations may occur which complete the transformation. Since TSH, through its influence on cell division, causes an expansion of the number of follicular cells at risk, it seems that the total chance of a spontaneous neoplastic event would increase as a function of the increase in cell number (assuming a constant probability of a spontaneous mutation per cell). According to this reasoning, treatments that increase thyroid cell number and increase mutations would be expected to enhance the carcinogenic process; there is some support for this position. For instance, regimens that combine a mutagenic agent (x-ray, genotoxic chemical) with an increased output of TSH (e.g.,iodide deficiency) result in an increase carcinogenic response. The same is true for chemicals that are both mutagenic and goitrogenic; for instance, 4,4'-methylenedianiline produces significant increases in thyroid tumors in males and females of both rats and mice.

If the above hypothesis is valid, it would seem that TSH is not a direct "initiator" of carcinogenesis, but rather it may allow cells to respond to other stimuli that finally complete the initiation stage. Once transformation occurs, TSH and other factors would be expected to promote carcinogenesis through their influence on cell division.

Experimental observations with a number of chemicals are consistent with the view that a major component in thyroid carcinogenesis results from prolonged exposure of the thyroid to elevated levels of TSH. To this end, most of the chemicals that have been shown to produce thyroid tumors in the NTP/NCI carcinogenesis bioassay program have been compounds from structural classes

(e.g., thionamides, aromatic amines) known to inhibit thyroid hormone synthesis. Another, nonspecific group of compounds that have been shown capable of causing thyroid neoplasia in laboratory animals are the inducers of hepatic mixed-function oxidases. These materials enhance the hepatic metabolism and biliary excretion of the thyroid hormones. The effects of both of these groups of compounds -- inhibition of thyroid hormone synthesis or increased thyroid hormone metabolism and elimination--result in decreased levels of circulating thyroid hormone and a consequent increase in the level of TSH. Genotoxic activity did not correlate with this type of thyroid carcinogenesis in any predictable way. Of the chemicals reviewed, only the aromatic amines showed genotoxic activity for a variety of end points.

Mechanisms other than TSH increases may influence thyroid tumor response. For those substances where gene mutations and structural chromosome aberrations may be induced, there is the possibility that a single or limited number of chemical-cell interactions may influence carcinogenesis.

In evaluating the nature of the dose-response relationship for chemicals that appear to have produced thyroid tumors via their influences on thyroid-pituitary status and an increase in TSH, several points should be kept in mind. Together these factors provide support for levels of TSH that are not associated with carcinogenic risk (i.e., subthresholds).

1. The proper maintenance of homeostatic control of circulating levels of the thyroid hormones requires some optimal, non-zero level of TSH.

2. TSH and the thyroid hormones must be continually replaced, since their residence in the body is finite (T_4: rat plasma $t^1/_2$ = 12-24 hours; human plasma $t^1/_2$ = 5-9 days) (see Thomas and Bell, 1982).

3. The feedback mechanism through which thyroid homeostasis is maintained depends ultimately on TSH-stimulated thyroid hormone synthesis by the thyroid

gland. The de novo synthesis of thyroid hormones is dependent on two separate receptor-mediated, dose-dependent steps. One of these occurs in the pituitary gland where receptors monitor circulating levels of the thyroid hormones and respond by releasing appropriate amounts of TSH; the second occurs in the thyroid gland itself where receptors respond to TSH. Hormone-receptor complexes are short lived and a supply of hormones must be present on an ongoing basis to interact with their receptors (T_3-receptor dissociation $t^1/_2$ = 15 min). In both the pituitary and the thyroid there exists a large number of receptors (several thousand per cell) for thyroid hormones and TSH, respectively, and the response of each gland is likely to be graded in nature and dependent on the number of receptors occupied at any one time. By analogy with other receptor-mediated reactions and from the information accumulated on the binding of T_3 by pituitary cell receptors, a large number of receptors must be occupied to elicit a response.

4. The effects of excessive TSH on thyroid cell histology/pathology (e.g., hypertrophy, hyperplasia) are reversible if the TSH stimulus is removed early in the process.

5. Thyroid cell proliferation and transformation involve several different steps and require a number of factors in addition to TSH. Some of the factors that may be operative work through receptors themselves and most likely require multiple site occupancy for effect.

6. Thyroid carcinogenesis seems to require long-term disruption in thyroid-pituitary status leading to elevated levels of TSH (and reduced levels of the thyroid hormones).

Humans appear to be quite similar to laboratory animals in their responses to goitrogenic stimuli. Thus, iodine deficiency, partial thyroidectomy (surgical or ^{131}I), and administration of antithyroid agents (e.g., thionamides) result in reduced thyroid hormones levels and increased levels of TSH, and can lead to

thyroid hypertrophy and hyperplasia. As in experimental animals thyroid enlargement and nodular lesions have been implicated as possible antecedents to thyroid cancer in humans.

In spite of these qualitative similarities, however, there is some evidence that humans may not be as sensitive quantitatively to thyroid cancer as experimental animal species. For instance, experimental animals readily respond to reduced iodide intake with thyroid cancer development. The case with humans is much less certain. Although there is profound hyperplasia with "adenomatous" changes, the case for malignant transformation is only suggestive and has not been demonstrated with any certainty. Even with congenital goiters where there are inherited blocks in thyroid hormone synthesis, only a few thyroid cancers have been reported in the literature. Humans also may be less sensitive to the effects of ^{131}I. Although the data are very soft, there does not seem to be any profound indication of a cancer problem in persons with Graves' disease where a significant proportion of patients have autoantibodies that stimulate the thyroid like TSH. In a like manner, these same patients treated with antithyroid compounds do not seem to show increases in thyroid cancer.

In contrast to the observations mentioned above, the finding of thyroid cancer in human autopsy studies in the United States is not unlike that seen in animal studies. For instance, about 1 percent of control Fischer 344 rats develop thyroid cancer over a lifetime, while autopsy prevalences of tumors in humans that were not noted during life range from 0.9 to 5.7 percent (about 2 percent average) in different studies. Few of the human tumors are manifest, since clinically significant thyroid tumors occur in only about 3 of 100,000 persons and constitute only about 0.5 percent of all cancer deaths.

B. SCIENCE POLICY

It is generally accepted that carcinogenesis is a long-term, complicated and multistep process with numerous causes. Although it is very difficult to prove that carcinogenesis proceeds via specific, discrete steps, in certain cases accumulated evidence becomes persuasive enough to presume that certain processes are operative, and this information can be used as the basis for an approach to estimate human cancer risk. This is the case for the induction of certain follicular cell neoplasms of the thyroid gland.

Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (e.g., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations and the rationale set out above in Section VII.A., the Agency concludes that:

1. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels;
2. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and

3. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance.

There are special considerations that must be addressed before applying this policy to any chemical substance that has produced thyroid tumors and is subject to review by the Agency. It is recognized that some thyroid tumors seem to arise from mechanisms other than thyroid-pituitary imbalance. It is also known that chemical substances may impact living cells in a number of different ways and, therefore, may be producing toxic effects by different mechanisms. Thus, two basic questions must be satisfactorily addressed in the risk assessment of chemicals under review in determining whether and how to apply the policy. The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents. Criteria for addressing these issues are developed below.

The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignation is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response. The guidance on how to proceed with the quantitation of risk varies with the category, as follows.

1. Threshold considerations should be applied in dose-response assessments for those chemical substances where (a) only thyroid tumors (and relevant pituitary tumors) have been produced; (b) the tumors can be

attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (c) potential mechanisms other than thyroid-pituitary imbalance (e.g., genotoxicity) can be disregarded.

2. Special attention should be given to chemicals (a) that have induced thyroid tumors (and relevant pituitary tumors) that may be due to thyroid-pituitary imbalance, and (b) where there is also evidence of either a genotoxic potential or the induction of neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.

3. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with the EPA Guidelines for Carcinogen Risk Assessment.

The application of this guidance is contingent upon the careful assessment of all information hearing on the carcinogenicity of each chemical subject to review. It calls for an evaluation of the types of thyroid (and pituitary) tumors and any other tumor types as well as preneoplastic and other toxicological lesions that are produced. It also requires a careful analysis of relevant mechanistic information bearing on the assessment of carcinogenicity. In certain cases data gaps may necessitate further testing and research before an assessment based on this policy can be completed. The remainder of this section will be devoted to a discussion of some of the factors that should be considered in the assessment of chemicals producing thyroid tumors.

One essential factor is whether the thyroid tumors can be attributed to disruption of thyroid-pituitary hormonal balance. In addressing whether this

is the case, the presence of several indicators should be considered.

1. Goitrogenic activity in vivo (i.e., thyroid follicular cell hypertrophy and hyperplasia)
2. Clinical chemistry indication of changes in thyroid and pituitary functional parameters (e.g., reduced thyroid hormone and increased TSH serum concentrations).
3. Specific evidence that the agent either reduces thyroid hormone synthesis (e.g., inhibits iodine uptake) or increases thyroid hormone clearance (e.g., enhances biliary excretion).
4. A progression of lesions under long-term exposure to an agent, showing cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia (benign and possibly malignant tumors).
5. Other studies bearing on the hypothesis that thyroid-pituitary imbalance may be operative, like reversibility of lesions following cessation of the treatment.
6. Structure-activity analysis of the agent under review to see if it belongs to a class of compounds that shows a correlation with the induction of thyroid tumors.

For each chemical that shows thyroid follicular cell carcinogenic effects, the above points are reviewed as a whole, and an overall judgment is made as to the likelihood the tumors may be due to a disruption in thyroid-pituitary status. Since the data base on chemicals will vary considerably, precise criteria as to what constitutes adequate evidence cannot be given, but at a minimum information from items 1, 2, and 3, with some indication of dose-response, are essential in making these judgments. In addition, several other of the above lines of evidence in support of the hypothesis are valuable, and while it is unlikely that one will ever amass direct proof of the hypothesis, enough supportive

information should be available so that the position is scientifically reasonable.

Another important point is the extent to which genotoxicity may account for the observed tumor effects. Short-term in vitro and in vivo tests for various end points, including gene and chromosomal mutations and DNA-damaging capability, should be reviewed to get an idea of the spectrum of effects that may be produced in somatic cells. 1/ It is recognized that a chemical seldom produces all positive or all negative responses in such tests; therefore, a case-by-case judgment must be made of the likelihood the chemical's carcinogenic effects may be due to its genotoxic activity. It should be pointed out that for the purposes of this policy, it is necessary to evaluate potential carcinogenic mechanisms and not just the correlation between short-term test results and carcinogenicity.

Certain short-term test end points have more intuitive relevance to carcinogenicity than others. End points such as gene and chromosomal mutations readily fit into what is known about carcinogenic mechanisms, whereas less can be said about the applicability of other end points like sister chromatid exchange or mitotic gene conversion. Even with mutations, there are different ways, at least theoretically, that chemicals might induce them and that may be relevant to dose-response considerations. It is conceivable that gene mutations arise from single (or a limited number of) chemical-cellular interactions, whereas at least two (and probably more) would be required for stable structural aberrations, and most likely many interactions would be needed to induce numerical chromosome aberrations.

1/ The Agency's Guidelines for Mutagenicity Risk Assessment should be consulted, but with the understanding that making a judgment of mutagenic risk to future generations (germ cell risk) involves an important aspect not relevant to carcinogenicity evaluation. Valuable reviews of short-term tests and testing results can be found in publications of the EPA Gene Tox program (U.S. EPA, 1988).

Neoplasms that occur in addition to thyroid follicular tumors (and relevant pituitary tumors) must be carefully evaluated as to mechanistic considerations. There is no a priori evidence that elevation in TSH serum concentration is associated with tumors at sites other than the thyroid. However, it is recognized that most organs of the body are responsive to thyroid hormone, and thus neoplastic development may in some way be modified under conditions that result in reduced circulating thyroid hormone concentration. This eventuality should be considered and evaluated. In addition, the role of target-organ toxicity, immunologic suppression and any other relevant biological properties of the chemical under study should be reviewed in assessing the significance of these tumors. Metabolic and pharmacokinetic considerations are also relevant.

The last major point in the evaluation of thyroid carcinogens is the way to quantitate carcinogenic risk when it is judged that the tumors are associated with thyroid-pituitary imbalance and threshold concepts apply. The traditional way the Agency has dealt with thresholds is to use a no-observed-adverse effect level (NOAEL) for the critical effect as a measure of potency and then to use uncertainty factors to estimate exposure levels (dose rates) where it is anticipated there will be little chance of risk in humans. An alternate means of expressing a degree of concern is to calculate a margin of exposure, the ratio of the NOAEL to anticipated human exposure. The larger the ratio, the less likely the exposure will be cause for concern, while the smaller the ratio, the greater the concern. Historically, the Agency has used threshold concepts to evaluate the risks related to target-organ and systemic toxicity and developmental

and reproductive toxicity. 2/

Based on current Agency practices, the procedures used for evaluating risks from systemic toxicants and other threshold-relevant end points may be employed. For those thyroid follicular cell tumors that are conceived as arising from an imbalance in the thyroid-pituitary axis, one needs to use toxicological parameters that give some indication that thyroid-pituitary homeostasis has been disrupted. End points that should be considered as bases of NOAELs include such things as increases in thyroid weight, decreases in circulating thyroid hormone, and increases in TSH concentration as a function of chemical dose. It is expected that these end points will show deviations from normal at doses lower than or equal to those showing increases in thyroid tumors. A NOAEL is determined for each meaningful toxicological end point, and the one from among those reviewed that demonstrates the lowest NOAEL is called the critical effect, that is, the most sensitive indicator of a pertubation in thyroid-pituitary balance. Either uncertainty factors or anticipated human exposure are used with this NOAEL to calculate measures associated with human risks. When chemically exposed groups of humans are available, clinical chemistry measurements (e.g., serum TSH concentrations) and other measures are useful in evaluating risks by comparing the distribution of values in this group as compared to a control group.

In evaluating thyroid follicular cell neoplasms under this policy, the risk assessment depends on full use of the available information. As indicated above,

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2/ Because the Agency recognizes that the traditional techniques are not necessarily the most sophisticated means of extrapolating risks, it is important to investigate alternative means of extrapolatinq risks in situations involving thresholds. Some of these alternatives are being considered for investigation or are already under development within the Agency. These include the use of a combination of high-to-low dose modeling and uncertainty factors and considerations of initiation-promotion phenomena in biologically based models. The Agency should actively pursue the application of some of these alternatives to the evaluation of human risks for thyroid "threshold" carcinogens in place of the traditional way the Agency has dealt with threshold considerations.

in any given organism, a carcinogen may act through more than one mechanism at one or multiple anatomical sites. Accordingly, while use of this policy may be appropriate for assessing certain thyroid follicular cell tumors, use of other models may be necessary to evaluate risks at other tumor sites observed in the same study, which may result in different risk estimates. It is incumbent upon the risk assessor to consider all relevant risk estimates in making the final judgments on the potential human risk related to exposure to the chemical being evaluated.

APPENDIX A

COMBINED TREATMENT STUDIES

Test Animal	Treatment A	Treatment B	Results	Reference
Wistar rat (female)	AAF (2.5 mg gavage, 4-6x for one week)	MTU (0.1 g/L in drinking water up to 21 wk)	Combined treatment showed multiple adenomas/gland. MTU alone caused hyperplasia or single tumors. AAF stated as having no tumor effect Combined treatment showed multiple adenomas when interval between treatments extended for 4-18 wk.	Hall, 1948
Lister rat (male & female)	AAF (100 mg/L in drinking water for 13 mo.	MTU (1 g/L in drinking water for 13 mo. concurrent with AFF).	Combined treatment showed more adenomas/gland than single treatment groups.	Doniach, 1950
Lister rat (male & female)	^{131}I (30 uCi, ip)	MTU (1 g/L in in drinking water for 15 mo)	Combined treatment produced more adenomas/gland and malignancies not seen in single treatment groups.	Doniach, 1953
Wistar rat (male)	X-rays (300 rad to neck)	MTU (1 g/L in drinking water for 15-18 mo)	Combined treatment increased incidence of tumor-bearing animals and malignancies that were not seen with single treatments.	Christov, 1975
Wistar rat (male)	DHPN (70 mg/100 g bw given sc once/wk for 4 or 8 wk)	Amitrole (2000 ppm in diet for 12 wk)	Amitrole after 4 wk of DHPN induced thyroid adenomas at 91% and carcinomas at 9%. No tumors with DHPN or amitrole alone. Amitrole accelerated development of adenomas and increased carcinomas after 8 wk of DHPN (no amitrole - 58% adenomas, 18% carcinomas; with amitrole - 100% adenomas, 42% carcinomas). No tumors with amitrole alone.	Hiasa et al., 1982a

(continued on the following page)

APPENDIX A. (continued)

Test Animal	Treatment A	Treatment B	Results	Reference
Wistar rat (male)	DHPN (70 mg/ 100 g bw given sc once/wk for for 4 or 6 wk)	PB (500 ppm in diet for 12 wk)	PB after 4 wk of DHPN induced thyroid adenomas at 66% and carcinomas at 10%. No tumors with DHPN or PB alone. PB after 6 wk of DHPN acclerated development of adenomas and induced carcinomas (no PB-23%) adenomas, no carcinomas; with PB-100% adenomas, 25% carcinomas; no tumors with PB alone).	Hiasa et al., 1982b
		BB (500 ppm in diet for 12 wk)	PB after 4 wk of DPHN induced thyroid adenomas (23%) but no carcinomas. No tumors with BB alone. BB after 6 wk of DHPN accelerated development of adenomas and induced a small number of carcinomas (no BB - 23% adenomas, no carcinomas; with BB - 45% adenomas, 10% carcinomas; no tumors with BB alone).	
Wistar rat (male)	DHPN (single sc dose of 280 mg/100 g bw)	PB (500 ppm in diet for 6, 12 or 19 wks)	PB for 12 or 19 wk after DHPN enhanced development of thyroid adenomas. PB for 19 wk after DHPN induced thyroid carcinomas at 12%. Not seen with DHPN alone. PB alone produced no tumors.	Hiasa et al., 1983
Wistar rat (male)	DHPN (single sc dose of 280 mg/100 g bw)	PTU (1500 ppm in diet for 19 wk)	PTU after DHPN enhanced development of thyroid follicular cell adenomas and induced carcinomas (no PTU - 19% adenomas, 0% carcinomas; with PTU - 100% adenomas, 52% carcinomas). PTU alone produced no tumors.	Kitahori et al., 1984.

(continued on the following page)

APPENDIX A. (continued)

Test Animal	Treatment A	Treatment B	Results	Reference
Wistar rat (male)	DHPN (single ip dose of 280 mg/100g bw)	MDA (1000 ppm in diet for 19 wk)	MDA after DHPN enhanced development of thyroid tumors and induced carcinomas (no MDA - 28% tumors, 0% carcinomas; with MDA - 90% tumors, 9.5% carcinomas). MDA alone produced no tumors.	Hiasa et al., 1984
F344/NCr rat (male)	NMU (single iv dose of 41.2 mg/kg bw)	Iodine deficient diet after 2 wk until 20 or 33 wk)	Iodine deficiency after NMU enhanced development of thyroid follicular cell adenomas and carcinomas (NMU alone - 10% adenomas at 20 wk and 70% adenomas at 33 wk, 10% carcinomas at 33 wk; NMU with iodine deficiency - 100% adenomas at 20 wk and 100% carcinomas at 33 wk; no tumors following iodine deficiency alone).	Ohshima and Ward, 1986
F344/NCr rat (male)	NMU (single iv dose of 41.2 mg/kg bw)	Iodine deficiency after 2 wk until 52 and 77 wk	Iodine deficiency after NMU enhanced development of the thyroid follicular cell carcinomas (NMU alone; 32% carcinomas at 52 wk; NMU with iodine deficiency, 90% at 52 wk). Iodine deficiency alone induced mostly thyroid adenomas and a few carcinomas (40% adenomas at 52 wk, 60% adenomas at 77 wk, and 10% carcinomas at 77 wk).	Ohshima and Ward, 1984
Wistar rat (female)	NMU (40 mg/kg bw by gavage for 3 days)	MTU [1 g/L in drinking water from 4 wk after NMU until death (60 wk)	Combined treatment resulted in appearance of thyroid follicular cell adenomas (within 13 wk) and carcinomas (after 16 wk) that metastasized to the lung (after 30 wk). No single treatment groups were included, and the fate of untreated controls was not described.	Schaffer and Muller, 1980

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APPENDIX A. (continued)

Test Animal	Treatment A	Treatment B	Results	Reference
F344 rat (female)	NMU (single iv dose of 50 mg/kg bw)	PTU (3, 10, and 30 mg/L in drinking water)	PTU after NMU induced development of thyroid adenomas and carcinomas (NMU alone - no tumors; with 3 mg/L PTU - 17% adenomas, 23% carcinomas; with 10 and 30 mg/L PTU - 100% carcinomas). No PTU alone group was included.	Milmore et al., 1982
		^{131}I (1 and 10 uCI)	No thyroid tumors.	
F344 rat (male)	NMU (20 mg/kg ip 2x/wk for 4 wk)	PB (0.05% in diet for 32 wk)	PB after NMU-induced thyroid papillary carcinomas. NMU alone did not induce tumors. PB was not tested alone.	Tsuda et al., 1983

KEY: AAF, 2-acetylaminofluorene; MTU, 4-methyl-2-thiouracil; DHPN, N-bis(2-hydroxypropyl)nitrosamine; amitrole, 3-amino-1,2,4-triazole; PB; phenobarbital; BB, barbital; PTU, propylthiouracil; MDA, methylenedianiline; NMU, N-methyl-N-nitrosourea.

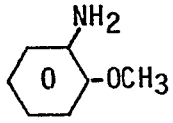
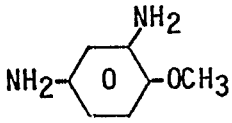
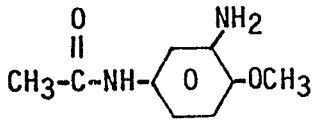
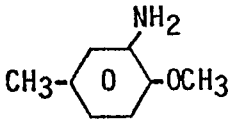
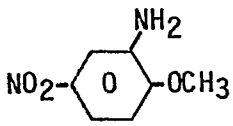
APPENDIX B

SINGLE RING AROMATIC AMINES

Several structurally related, single-ring aromatic amines have been tested for carcinogenicity and are illustrated in the accompanying table. Of the 11 structural analogues, only o-anisidine, (no. 1), 2,4-diaminoanisole (no. 2), 3-amino-4-ethoxyacetanilide (no. 3), and HC Blue No. 1 (no. 9) were positive for thyroid tumors.

Although the first three chemicals share amino and methoxy substituents in the ortho position on the ring, other tested chemicals with this conformation (no. 4, no. 5) did not produce thyroid tumors. Both chemicals, no. 2 and no. 3, have amino groups in the meta position on the ring; however, compound no. 8, which also has this configuration, lacked thyroid tumor activity. Chemicals no. 2 and no. 3 also shared amino and methoxy groups in the para positions; compounds no. 6 and 7 with these constituents were negative for thyroid tumors. Likewise, for HC Blue No. 1 (no. 9), which showed a thyroid tumor response in the NTP bioassay, structural analogues no. 10 and 11 failed to show this response. Thus, it is not readily apparent which, if any, substitutions on the ring may impact thyroid tumor activity.

SINGLE RING AROMATIC AMINES:
STRUCTURE-ACTIVITY RELATIONSHIPS AMONG CHEMICALS TESTED BY THE NCI/NTP

	Thyroid Tumors				Other Tumors			
	Rat		Mouse		Rat		Mouse	
	M	F	M	F	M	F	M	F
1. o-anisidine	+	-	-	-	bladder kidney	bladder	bladder	bladder
								
2. 2,4-diaminoanisole	+	+	+	+	skin liver	skin liver	-	liver
								
3. 3-amino-4-ethoxy-acetanilide	-	-	+	-	-	-	-	-
								
4. p-cresidine	-	-	-	-	bladder nasal liver	bladder nasal -	bladder - -	bladder - liver
								
5. 5-nitro-o-anisidine	-	-	-	-	skin zymbal	skin zymbal clitoral gland	- - liver	- - -
								

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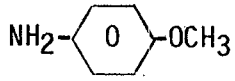
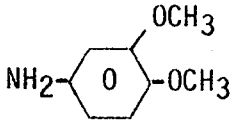
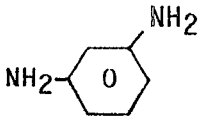
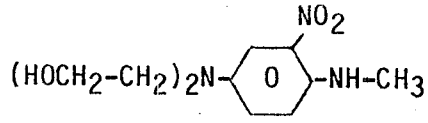
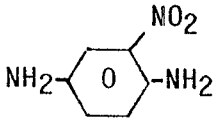
APPENDIX B. (continued)

	Thyroid Tumors				Other Tumors			
	Rat		Mouse		Rat		Mouse	
	M	F	M	F	M	F	M	F
6. p-anisidine 	-	-	-	-	-	-	-	-
7. 3,4-dimethoxyaniline 	-	-	-	-	-	-	-	-
8. m-diphenyleneaxine 	-	-	-	-	-	-	-	-
9. HC Blue No. 1 	-	-	+	-	liver	lung	liver	liver
10. p-phenylenediamine 	-	-	-	-	-	-	-	-
11. 2-nitro-p-phenylene-diamine 	-	-	-	-	-	-	-	-

APPENDIX C

GENOTOXICITY: ETHYLENE THIOUREA

1. GENE MUTATIONS

A. BACTERIA

Salmonella (Ames)		Reported Effect	Reference
G46		(w)	Seiler, 1974
G46	N-nitrosoethylenethiourea	(+)	Seiler, 1977
multiple strains	($-NO_2^-$)	(w)	Shirasu et al., 1977
	($+NO_2^-$)	(+)	
mouse/rat host mediated	($-NO_2^-$)	(-)	
G46	($+NO_2^-$)	(+)	
multiple strains		(+) TA 1530 only	Schupbach and Hummler, 1977
mouse host mediated G46, TA 1530		(+) TA 1530 only	
multiple strains		(+) in all	Anderson and Styles, 1978
TA 1950	($-NO_2^-$)	(w)	Autio et al., 1982
	($+NO_2^-$)	(+)	
mouse host mediated (TA 1950)	($-NO_2^-$)	(w)	
	($+NO_2^-$)	(+)	
multiple strains		(w) TA 1535 only	Moriya et al., 1983
mouse host mediated (TA 1950)	($-NO_2^-$)	(-)	Braun et al., 1977
	($+NO_2^-$)	(+)	
multiple strains/replications in different labs		(w) TA 1535 (-) all others	Mortelmans et al., 1986
multiple strains/replications in different labs		(-)	Bridges et al., 1981

E. coli

WP2	($-NO_2^-$)	(-)	Shirasu et al., 1977
	($+NO_2^-$)	(+)	
WP2		(-)	

(continued on following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

B. EUKARYOTIC MICROORGANISMS			
Saccharomyces (XV 185-14C)	(+)	requires S9	Mehta and vonBorstel, 1981
Schizosaccharomyces	(-)		Loprieno, 1981
C. HIGHER EUKARYOTES			
Mouse lymphoma cells (TK)	(-)		Jotz and Mitchel, 1981
Mouse lymphoma cells	(+)		NTP, 1986
Chinese hamster ovary (several loci)	(-)		Carver et al., 1981
Drosophila XLRL	(-)		Valencia and Houtchens, 1981
Drosophila XLRL	(-) (?)	injection feeding	Woodruff et al., 1985
Drosophila XLRL	(+)		NTP, 1986
2. CHROMOSOME EFFECTS			
A. NUMERICAL ABERRATIONS			
Saccharomyces mitotic aneuploidy	(+)		Parry and Sharp, 1981
Mouse micronucleus (see B, below)			
B. STRUCTURAL ABERRATIONS			
Chinese hamster ovary cells	(-)		Shirasu et al., 1977
Chinese hamster ovary cells	(-)		Natarajan and vanKesteren-van Leeuwen, 1981
Chinese hamster ovary cells	(-)		NTP, 1986
Mouse micronucleus (B6C3F1)	(-)		Salamone et al., 1981
Mouse micronucleus (ICR)	(-)		Kirkhart, 1981
Mouse micronucleus (CD-1)	(-)		Tsuchimoto and Matter, 1981

(continued on following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

Mouse micronucleus	($-NaNO_2$) ($+NaNON_2$)	(-) (+)	Seiler, 1975
Mouse micronucleus		(-)	Schupbach and Hummler, 1977
Mouse dominant lethal		(-)	Shirasu et al., 1977
Mouse dominant lethal		(-)	Schupbach and Hummler, 1977
Mouse dominant lethal		(-)	Schupbach and Hummler, 1977
Mouse dominant lethal	(+ $NaNO_2$)	(+) preimplantation loss	Teramoto et al., 1978
		(-) postimplantation loss	
Chinese hamster bone marrow	(+$NaNO_2$)	(+)	Seiler, 1977
Rat bone marrow		(-)	Shirasu et al., 1977
Drosophila reciprocal translocation		(-)	NTP, 1986
C. SISTER CHROMATID EXCHANGES			
Chinese hamster ovary cells		(-)	Evans and Mitchel, 1981
Chinese hamster ovary cells		(-)	Natarajan and vanKesteren - van Leeuwen, 1981
Chinese hamster ovary cells		(-)	Perry and Thomson, 1981
Chinese hamster ovary cells		(-)	NTP, 1986
Mouse in vivo (CBA/J)		(-)	Paika et al., 1981
3. DNA DAMAGE			
B. subtilis (rec)		(w) without S9 (-) with S9	Kada, 1981
E. coli (pol A)		(-)	Green, 1981

(continued on following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

E. coli (rec)	(+)	with S9	Ichinotsubo et al., 1981
E. coli (rec, pol A)	(-)		Tweats, 1981
E. coli (pol A)	(w)	without S9	Rosenkranz et al., 1981
	(-)	with S9	
E. coli (lambda induction)	(+)		Thomson, 1981
Saccharomyces mitotic cross-over	(-)		Kassinova et al., 1981
Saccharomyces mitotic gene conversion	(-)		Jagannath et al., 1981
Saccharomyces mitotic gene conversion	(-)		Zimmemann and Scheel, 1981
Saccharomyces (JDI) mitotic gene conversion	(+)	without S9	Sharp and Perry, 1981a
Saccharomyes (RAD) differential growth			Sharp and Perry, 1981b
Unscheduled DNA synthesis WI-38 cells	(-)		Robinson and Mitchell, 1981
Human fibroblasts	(-)		Agrelo and Amos, 1981
Mouse sperm morphology	(-)		Wyrobek et al., 1981
Mouse sperm morphology	(-)		Tophan, 1980

4. IN VITRO TRANSFORMATION

Baby hamster kidney (BHK 21)	(+)	Daniel and Dehne, 1981
Baby hamster kidney (BHK 21)	(+)	Styles, 1981
Syrian hamster embryo, adenovirus infected (SHE-SA7)	(-)	Hatch et al., 1986

Key: (+) postive
(w) weak positive
(?) equivocal
(-) negative

APPENDIX D

GENOTOXICITY: 4,4'-OXYDIANILINE

1. GENE MUTATION

A. BACTERIA

Salmonella (Ames)	Reported Effect	Reference
TA 98 TA 100	(+) requires S9 (+) assayed only in presence of S9	Lavoie et al., 1979
TA 98 TA 100	(w) requires S9 (+) requires S9	Parodi et al., 1981
TA 98 TA 100	(+) requires S9 (+) requires S9	Tanaka et al., 1985
TA 97 TA 98 TA 100 TA 1535 TA 1537	(+) requires S9 (+) requires S9 (+) with or without S9 (+) requires hamster S9 (+) assayed only with S9; requires hamster S9	NTP, 1987 (personal communication Dr. Errol Zeiger)

B. EUKARYOTES

Mammalian cells in culture Mouse lymphoma	(+)	NTP, 1986

2. CHROMOSOME EFFECTS

Chinese hamster ovary cells structural chromosome aberrations	(+)	NTP, 1986
sister chromatid exchanges	(+)	
Rat bone marrow sister chromatid exchanges	(-)	Parodi et al., 1983

(continued on following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

3. DNA DAMAGE		
Unscheduled DNA synthesis (rat hepatocytes)		
in vivo	(-)	Mirsalis et al., 1983
in vitro	(-)	
4. IN VITRO TRANSFORMATION		
Syrian hamster embryo cells	(?)	Tu et al., 1986
Enhancement of virus infected transformation of Syrian hamster embryo cells	(+)	Hatch et al., 1986

Key: (+) - positive
(w) - weak positive
(?) - equivocal
(-) - negative

APPENDIX E

GENOTOXICITY: AMITROLE

1. GENE MUTATIONS

A. BACTERIA		Reported Effect	Reference
Salmonella (Ames)		(-)	See multiple bacterial tests summarized in Bridges et al., 1981
		(-)	McCann and Ames, 1976
TA 1950, mouse host mediated	($-NO_2^-$)	(-)	Braun et al., 1977
	($+NO_2^-$)	(w)	
		(-)	Dunkel, 1979
		(-)	Rosenkranz and Poirier, 1979
		(-)	Moriya et al., 1983
		(-)	NTP, 1986
E. coli			
(WP2uvrA (P))		(+)	Venitt and Crofton-Sleigh, 1981
(WP2uvrA)		(-)	Matsushima et al., 1981
(WP2uvrA/pKM101)		(-)	Matsushima et al., 1981
Streptomyces		(w)	Carere et al., 1978
B. EUKARYOTIC MICROORGANISMS			
Saccharomyces (RV)		(-)	Mehta and vonBorstel, 1981

(continued on following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

C. HIGHER EUKARYOTES		
Drosophila XLRL	(-)	Laamanen et al., 1976
	(-)	Vogel et al., 1980
	(-)	Vogel et al., 1981
	(?)	NTP, 1986
	(feeding, ?; injection, -)	Woodruff et al., 1985
Mouse lymphoma L5178Y cells (TK)	(-/-/-)	NTP, 1986
Syrian hamster embryo cells		
(ouabain)	(+)	Tsutsui et al., 1984
(6-thioguanine)	(+)	Tsutsui, et al., 1984
2. CHROMOSOME EFFECTS		
A. NUMERICAL ABERRATIONS		
Saccharomyces (D6)	(-)	Parry and Sharp, 1981
Aspergillus mitotic nondisjunction	(w)	Bignami et al., 1977
Drosophila sex chromosome nondisjunction	(-)	Laamanen et al., 1976
B. STRUCTURAL ABERRATIONS		
Human lymphocytes in vitro	(-)	Meretoja et al., 1976
Mouse micronucleus (B6C3F1)	(-)	Salomone, et al., 1981
(CD-1)	(-)	Tsuchimoto and Matter, 1981
Mouse dominant lethal (Ha, 1 CR)	(-)	Food and Drug Res., 1978

(continued on the following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

C. OTHER EFFECTS		
sister chromatid exchange		
(CHO)	(+)	Perry and Thomson, 1981
(CHO)	(+)	NTP, 1986
3. DNA DAMAGE		
Bacillus subtilis		
Rec	(+)	Kada, 1981
E. coli		
Rec	(-)	Green, 1981
Rec	(-)	Ichinotsubo et al., 1981
Rec	(-)	Mamber et al., 1983
Rec	(-)	Tweats, 1981
PolA	(-)	Rosenkranz et al., 1981
Lambda prophage induction	(-)	Thomson, 1981
Saccharomyces cerivisiae		
(D3) mitotic cross over	(-)	Simmon, 1979
(race XII) mitotic cross over	(-)	Kasinova et al., 1981
(D4) mitotic gene conversion	(-)	Jagannath et al., 1981
(D7) mitotic gene conversion	(-)	Zimmerman and Scheel 1981
(JD1) mitotic gene conversion	(+)	Sharp and Perry, 1981 1981a

(continued on the following page)

KEY: (+) positive
(w) weak positive
(?) equivocal
(-) negative

(RAD) cell growth	(+)	Sharp and Perry, 1981b
Aspergillus mitotic cross	(w)	Bignami et al., 1977
Unscheduled DNA synthesis (HeLa)	(+)	Martin and McDermid, 1981
MLV integration enhancement (C3H2K)	(-)	Yoshikur and Matsushima, 1981
Mouse sperm head abnormality	(-)	Tophan, 1980
4. IN VITRO TRANSFORMATION		
Syrian hamster embryo cells	(+)	Dunkel et al., 1981
	(+)	Tsutsui, et al., 1980
Baby hamster kidney cells (BHK)	(+)	Styles, 1980
	(+)	Styles, 1981
Rat Embryo cells	(-)	Daniel and Dehnel, 1981
(Rauscher murine leukemia virus infected)	(+)	Dunkel et al., 1981
	(+)	NTP, 1983

KEY: (+) - positive
(w) - weak positive
(?) - equivocal
(-) - negative

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