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ASSESSMENT OF THYROID FOLLICULAR CELL TUMORS
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ABSTRACT

The U.S. Environmental Protection Agency (U.S. EPA) conducts risk assessments on chemicals for carcinogenicity under the guidance provided in its cancer risk assessment guideli (U.S. EPA, 1986a; 1996). From time to time scientific developments prompt the Agency to reexamine procedures that are generally applied. That is the case with the review of some chemicals that have produced thyroid follicular cell tumors in experimental animals. The purp the Risk Assessment Forum report Assessment of Thyroid Follicular Cell Tumors (EPA/630/R-97-002) is to describe the procedures U.S. EPA will use to evaluate these tumor the data that are needed to make these judgments.

The Report presents science policy guidance that describes the procedures the Agency use in the evaluation of potential human cancer hazard and dose-response assessments from chemicals that are animal thyroid carcinogens. The Forum Report describes when, under clear specified conditions, chemical carcinogenesis in thyroid follicular cells can be analyzed as a nonlinear phenomenon, rather than assuming low dose linearity as EPA customarily does for carcinogenic compounds. Four hypothetical case studies are summarized which illustrate how evaluate toxicological data and make hazard and dose-response estimation choices. The proce and considerations developed in the Forum Report embody current scientific knowledge of th carcinogenesis and evolving science policy. Should significant new information become availa the Agency will update its guidance accordingly.

Send comments/questions to ncea.webmaster@epamail.epa.gov.

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March 1998

ASSESSMENT OF THYROID FOLLICULAR CELL TUMORS

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CONTENTS

Lists of Tables and Figures v

Peer Reviewers vi

Preface viii

EXECUTIVE SUMMARY 1

1. SUMMARY OVERVIEW OF THYROID CARCINOGENESIS 5
   1.1. ROLE OF TSH IN RODENT CARCINOGENESIS 7
   1.2. GENETIC INFLUENCES 7
   1.3. POSSIBLE MECHANISTIC STEPS 9
   1.4. HUMAN THYROID CARCINOGENESIS 11

2. SCIENCE POLICY GUIDANCE 16
   2.1. SCIENCE POLICY STATEMENTS 16
   2.2. EVIDENCE FOR ANTITHYROID ACTIVITY 19
      2.2.1. Data Needs 19
      2.2.2. Increases in Cellular Growth 20
      2.2.3 Hormone Changes 20
      2.2.4. Site of Action 21
         2.2.4.1. Intrathyroidal 21
         2.2.4.2. Extrathyroidal 21
      2.2.5. Dose Correlations 24
      2.2.6. Reversibility 24
      2.2.7. Lesion Progression 24
      2.2.8. Structure-Activity Analysis 24
      2.2.9. Other Studies 24
   2.3. OTHER MODES OF CARCINOGENIC ACTION 25
   2.4. DOSE-RESPONSE CONSIDERATIONS 25
      2.4.1. Thyroid Tumor High-to-Low Dose Extrapolation 26
         2.4.1.1. Biologically Based 26
         2.4.1.2. Nonlinear 26
         2.4.1.3. Low-Dose Linear 28
         2.4.1.4. Low-Dose Linear and Nonlinear 29
      2.4.2. Endpoints to be Employed 29
      2.4.3. Estimation of Points of Departure 29
      2.4.4. Interspecies Extrapolation 30
      2.4.5. Human Intraspecies Evaluations 31

3. HIGHLIGHTS OF CASE STUDIES 34
3.1. COMPOUND 1: A THIONAMIDE THAT AFFECTS THE SYNTHESIS OF ACTIVE THYROID HORMONE; THYROID PEROXIDASE AND 5'-MONODEIODINASE INHIBITION 34
3.2. COMPOUND 2: A CHLORINATED CYCLIC HYDROCARBON THAT MAY INFLUENCE THE THYROID THROUGH EFFECTS ON THE LIVER; SIGNIFICANT DATA GAPS 35
3.3. COMPOUND 3: A BIS-BENZENAMINE THAT PRODUCES THYROID AND LIVER TUMORS; ANTITHYROID AND MUTAGENIC EFFECTS 36
3.4. COMPOUND 4: A NITROSAMINE THAT IS MUTAGENIC AND HAS NO ANTITHYROID EFFECTS 36

REFERENCES 37

APPENDICES:

A. CASE STUDIES OF COMPOUNDS

B. SYNOPSIS OF AGENTS AFFECTING THE THYROID

C. SCIENTIFIC FINDINGS FROM 1988 EPA REVIEW DOCUMENT

D. A REVIEW OF RECENT WORK ON THYROID REGULATION AND THYROID CARCINOGENESIS BY GORDON HARD

LIST OF TABLES

Table 1. Carcinogenic influences on the rodent thyroid 9

Table 2. Possible molecular events in human thyroid (follicular) carcinogenesis 11

Table 3. Carcinogenic influences on the human thyroid 13

Table 4. Inter- and intraspecies differences 14

Table 5. Default dose-response procedures for thyroid carcinogens 19

Table 6. Data demonstrating antithyroid activity 20

Table 7. Case study summaries: dose-response assessments 34

LIST OF FIGURES

Figure 1. Hypothalamic-Pituitary-Thyroid Axis 6

Figure 2. Antithyroid Effects Influencing Thyroid Carcinogenesis 23

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PREFACE

The U.S. Environmental Protection Agency (U.S. EPA) conducts risk assessments on chemicals for carcinogenicity under the guidance provided in its cancer risk assessment guidelines (U.S. EPA, 1986a; 1996). From time to time scientific developments prompt the Agency to reexamine procedures that are generally applied. That is the case with the review of some chemicals that have produced thyroid follicular cell tumors in experimental animals. The purpose of this document is to describe the procedures U.S. EPA will use to evaluate these tumors and the data that are needed to make these judgments.

The current 1986 EPA cancer assessment guidelines provide direction for performing hazard and dose-response assessments for carcinogenic substances. The guidelines generally operate on the premise that findings of chemically induced cancer in laboratory animals signal potential hazards in humans. Likewise, for dose-response analyses, the guidelines first call for use of the most biologically appropriate means for dose extrapolation. In the absence of such knowledge, assessors are directed toward the use of a default science policy position, a low-dose linear procedure. The National Research Council in their report *Science and Judgment in Risk Assessment* (NRC, 1994) emphasized that well designed guidelines should permit acceptance of new evidence that differs from what was previously perceived as the general case, when scientifically justifiable. In keeping with this principle, the NRC recommended that EPA be more precise in describing the kind and strength of evidence that it will require to depart from a default option and which procedures will be applied in such situations.

The scientific analysis and science policy statement in this report apply only to tumors involving follicular cells of the thyroid gland. This report does not analyze or address comparable issues for other endocrine organs. Each one will generally need to be evaluated on its own merits (Iatropoulos, 1993/94; Capen et al., 1995).

The present report responds to the EPA policy concerning risks to infants and children (U.S. EPA, 1995). It finds that children are more susceptible than adults to the thyroid carcinogenic effects of ionizing radiation, and mutagenic thyroid carcinogenic chemicals should be reviewed to determine whether children may be more sensitive than adults. There is no indication that disruption of thyroid-pituitary status may lead to differential age sensitivity.

In 1988, the Agency developed a review of the existing science on thyroid follicular cell carcinogenesis and a draft science policy position covering the evaluation of chemicals that have induced thyroid tumors in experimental animals. The EPA Science Advisory Board approved the science review and tentatively embraced the policy position that some thyroid tumors could be

assessed using nonlinear considerations. However, they recommended that the Agency (a) articulate more clearly the steps that lead to the use of nonlinear considerations in assessments and (b) illustrate, using case studies, the ways EPA would evaluate data on animal thyroid carcinogens and make projections of anticipated human risk and dose-response assessments from chemicals that are animal thyroid carcinogens.

The present document responds to the comments of the Science Advisory Board. After an abbreviated overview of thyroid carcinogenesis, the paper presents science policy guidance that describes the procedures the Agency will use in the evaluation of potential human cancer hazard and dose-response assessments from chemicals that are animal thyroid carcinogens. Four hypothetical case studies are summarized which illustrate how to evaluate toxicological data and make hazard and risk estimation choices; the case studies are presented in full in Appendix A. Some of the chemical and functional classes and individual chemicals that have produced effects on the thyroid are included in Appendix B. The overview highlights the knowledge about thyroid carcinogenesis, but it does not contain an in depth scientific review of the literature. As support for the overview and as background for the science policy, the reader is referred to the scientific findings laid out in the 1988 EPA review document and since published (Appendix C) and to an update of some of the significant findings that have been published since the 1988 EPA review (Appendix D). The recent scientific literature further supports this science policy.

EXECUTIVE SUMMARY

Default assumptions that in the absence of relevant data help guide EPA in the use of animal data in evaluating potential human hazards and risks include the following: (a) tumors in animals are indicators of potential carcinogenic hazards in humans; (b) humans are generally as sensitive or more sensitive to effects of chemicals than are test animals; and (c) for carcinogens, dose and response maintain a linear relationship from high dose to zero dose, based on an assumption that chemicals act by affecting DNA directly to cause mutations. This paper reviews the evidence concerning these presumptions for thyroid follicular cell tumors and indicates that they do not entirely hold for some thyroid carcinogens. Data requirements for substantiating and describing thyroid effects of a chemical are elaborated.

Tumors of thyroid gland follicular cells are fairly common in chronic studies of chemicals in rodents. Experimental evidence indicates that the *mode of action* for these rodent thyroid tumors involves (a) changes in the DNA of thyroid cells with the generation of mutations, (b) disruption of thyroid-pituitary functioning, or (c) a combination of the two. The only verified cause of human thyroid cancer is ionizing radiation, a *mutagenic* insult to which children are more sensitive than adults.

Thyroid hormone from the thyroid gland helps to set the metabolic rate of cells throughout the body. Too little or too much thyroid hormone is associated with disease, hypothyroidism and hyperthyroidism, respectively. Thyroid hormone production and thyroid cell division are regulated by a negative feedback loop from the pituitary gland. Whenever the pituitary detects too much circulating levels of thyroid hormone, it reduces output of thyroid-stimulating hormone (TSH). When the pituitary perceives too little circulating thyroid hormone —whether due to decreased synthesis or increased metabolism and excretion of thyroid hormone from the body—the pituitary increases the output of TSH. TSH goes to the thyroid and stimulates the production of more thyroid hormone by existing cells or increases cell division in the thyroid to help meet the additional demands for thyroid hormone production.

Treatments of rodents that cause *thyroid-pituitary disruption* result in chronic reduction in circulating thyroid hormone levels, increase in TSH levels and the development of increased cell division, increased size and numbers of thyroid cells, increased thyroid gland weight and, finally, tumors of the thyroid. In some cases, there is also an increase in tumors of the pituitary cells that produce TSH. Cessation of treatment early in the process before tumor development results in reversal of processes back towards normal.

Qualitatively, it is not known whether humans are susceptible, as are rodents, to the development of thyroid cancer from thyroid-pituitary disruption. Those human conditions that

are known—namely iodide deficiency, congenital inability to synthesize thyroid hormone, and Graves' disease—are difficult to interpret regarding the influence of thyroid growth on thyroid cancer. The first two conditions result in situations like those found in rodents treated with chemicals that lead to thyroid-pituitary disruption. Like rodents, these individuals develop decreased thyroid hormone and increased TSH levels, increased cell division and thyroid gland enlargement, but cancer does not necessarily follow. Some studies of persons in iodide deficient areas show an association with thyroid cancer; other equally well conducted studies do not, and there have only been a few reported cancer cases in persons with an inability to synthesize thyroid hormone. Persons with Graves' disease, an autoimmune disorder, demonstrate antibodies against the TSH receptor which stimulate thyroid cell growth as does TSH itself. It has not been resolved whether persons with Graves' disease demonstrate an increased incidence of thyroid cancer or the clinical course is more aggressive when they develop cancer. *Quantitatively*, if humans develop cancer through thyroid-pituitary disruption, it appears that humans are less sensitive to the carcinogenic effects than are rodents. Rodents show significant increases in cancer with thyroid-pituitary disruption; humans show little, if any. However, given the data at hand and the questions that still remain unanswered, it seems that the finding of thyroid tumors in experimental animals cannot be totally dismissed as a hazard indicator for humans.

Using the current understanding of thyroid carcinogenesis, the EPA adopts the following *science policy* for interpreting data on this process in experimental animals:

1. It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid.
2. In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. This is a conservative position when thyroid-pituitary disruption is the sole mode of action, because rodents appear to be more sensitive to this carcinogenic mode of action than humans. When the thyroid carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than adults needs to be evaluated on a case by case basis.
3. Based on data and mode of action information on a chemical that has produced thyroid tumors, a judgment will be made concerning the applicability of the generic EPA presumption that dose and response maintain linearity from high dose to zero dose as follows:
    a. A linear dose-response procedure should be assumed when needed experimental data to understand the cause of thyroid tumors are absent and the mode of action is unknown.

b. A linear dose-response procedure should be assumed when the mode of action underlying thyroid tumors is judged to involve mutagenicity alone.

c. A margin of exposure dose-response procedure based on nonlinearity of effects should be used when thyroid-pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors. Thyroid-pituitary perturbation is not likely to have carcinogenic potential in short-term or highly infrequent exposure conditions. The margin of exposure procedure generally should be based on thyroid-pituitary disruptive effects themselves, in lieu of tumor effects, when data permit. Such analyses will aid in the development of combined noncancer and cancer assessments of toxicity. Results of the margin of exposure procedure will be presented in a way that supports risk management decisions for exposure scenarios of differing types (e.g., infrequent exposure, short durations).

d. Consistent with EPA risk characterization principles, both linear and margin of exposure considerations should be assumed when both mutagenic and thyroid-pituitary disruption modes of action are judged to be potentially at work. The weight of evidence for choosing one over the other should also be presented. The applicability of each to different exposure scenarios should be developed for risk management consideration.

e. When supported by available data, biologically based dose-response modeling may be conducted. This is the preferred approach when detailed data are available to construct such a model.

4. Adverse rodent noncancer thyroid effects (e.g., thyroid gland enlargements) following short- and long-term reductions in thyroid hormone levels are presumed to pose human noncancer health hazards.

5. Dose-response relationships for neoplasms other than the thyroid (or pituitary) will be evaluated using mode of action information bearing on their induction and principles laid out in current EPA cancer risk assessment guidelines.

Application of the science policy guidance for chemicals that have produced thyroid tumors in rodents is dependent on the generation of mode of action information regarding potential mutagenic influences on the DNA and whether there are changes in thyroid-pituitary functioning. Mutagenic influences are evaluated by short-term tests for gene and structural chromosome mutations and other tests.

Influences on thyroid-pituitary functioning are evaluated in eight different areas: data on five lines of evidence are required to evaluate this mode of action; three are desirable. Required is

information on increases in follicular cell size and number, changes in thyroid and pituitary hormones, knowledge of where the chemical affects thyroid functioning, correlations between doses producing thyroid effects and cancer, and reversibility of effects when chemical dosing ceases. Desirable information consists of knowledge of progression of lesions over time, chemical structure-activity relationships, and various other investigations (e.g., initiation-promotion studies).

Dose-response relationships will incorporate biologically based models when sufficient data are available. In their absence, linear, margin of exposure or both procedures will be employed, using extant mode of action information. The first step is to model existing data down to the point where information is generally no longer reliable, the *dose point of departure*. These include estimates of a dose producing 10% thyroid tumor incidence and values using noncancer effects like TSH levels, thyroid weight and other parameters. Concern for human exposure ("risk") using a *margin of exposure*, involves calculation of the ratio of a dose point of departure to an anticipated human exposure level. The larger the margin, the lower the concern for exposure. In cases where *linear* considerations are applied, a straight-line extrapolation of tumor incidence is made from the dose point of departure (e.g., 10% tumor incidence level) to the origin.

The procedures and considerations developed in this report embody current scientific knowledge of thyroid carcinogenesis and evolving science policy. Should significant new information become available, the Agency will update its guidance accordingly.

1. SUMMARY OVERVIEW OF THYROID CARCINOGENESIS

Circulating thyroid hormone determines the level of operation of most cells of the body (Brent, 1994); too much or too little hormone results in disease. Control of the concentration of this endocrine hormone in the blood is regulated mainly by a negative feedback involving three organs: the thyroid gland, which produces thyroid hormone, and the pituitary gland and hypothalamus, which respond to and help maintain optimal levels of thyroid hormone (figure 1).

The hypothalamus stimulates the pituitary through thyrotropin-releasing hormone (TRH) to produce TSH, which then prompts the thyroid to produce thyroid hormone. The stimulated thyroid actively transports inorganic iodide into the cell; it converts it to an organic form and then into thyroid hormone molecules that can influence target organs throughout the body. Thyroid hormone, in tissues peripheral to the thyroid, can be converted from a less active thyroxine (T_4) to a more active triiodothyronine (T_3) form. Thyroid hormone also is metabolized by the liver, largely by conjugation reactions, and excreted into the bile.

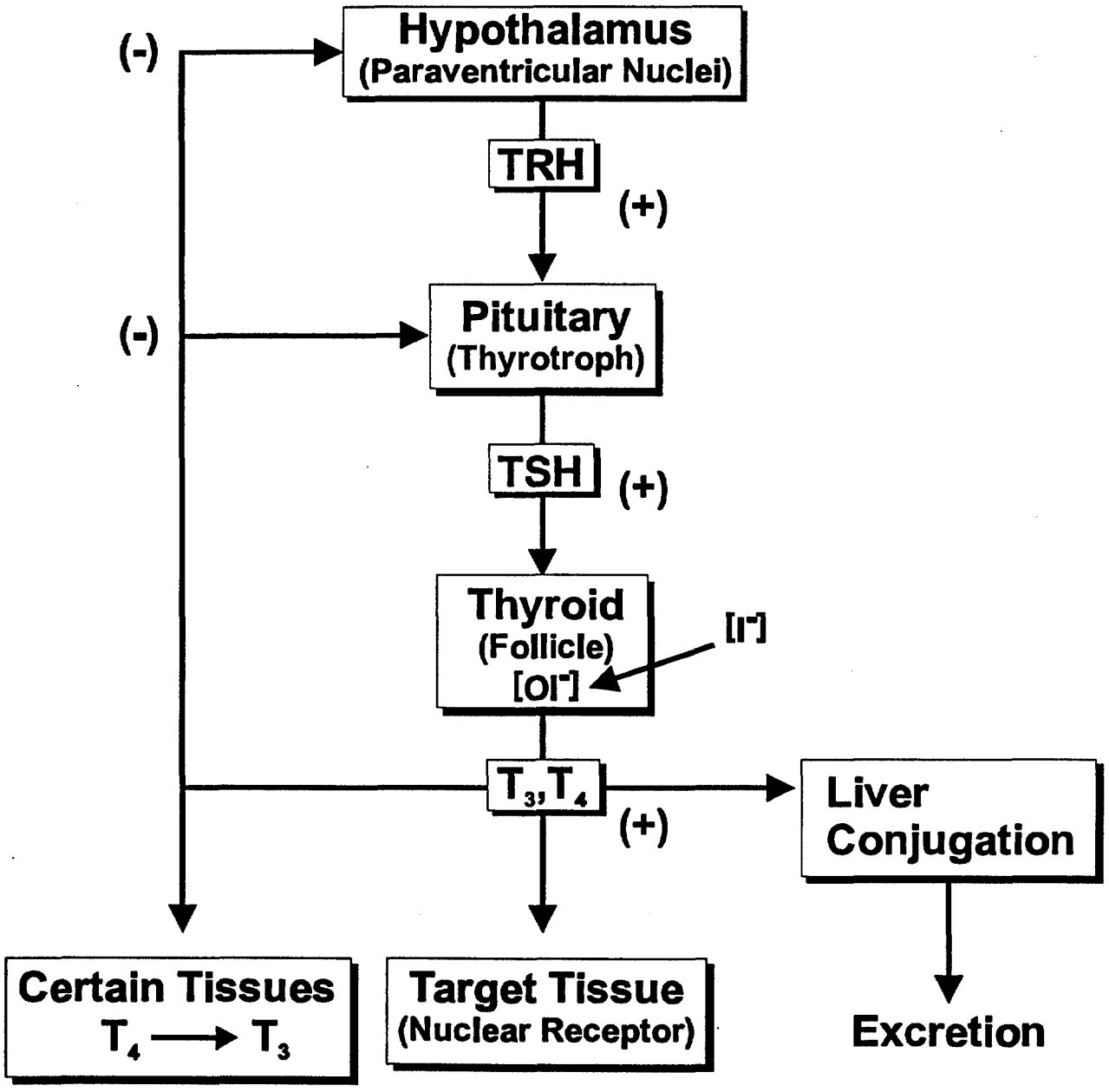
Cells in the hypothalamus and pituitary gland respond to levels of circulating thyroid hormone, such that when thyroid hormone levels are high there is a signal to reduce the output of TRH and TSH. Similarly, when thyroid hormone levels are reduced, the pituitary is prompted to deliver more TSH to the thyroid gland to increase the output of thyroid hormone. This negative feedback loop helps the body to respond to varying demands for thyroid hormone and to maintain hormone homeostasis. Circulating T_4, T_3, and TSH can readily be monitored in experimental animals and humans and serve as biomarkers of exposure and effect of agents that disrupt thyroid-pituitary status.

In higher organisms, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet the demand. With increased need, as a result of certain chemical exposures or iodide deficiency, the thyroid responds by increasing the size (hypertrophy) and number (hyperplasia) of thyroid follicular cells to enhance hormone output. With continued TSH stimulation, there is actual enlargement of the thyroid gland (goiter) and, at least in rodents, eventually neoplasia of the thyroid follicular cells. Because TSH-producing pituitary cells also are stimulated, they too sometimes undergo hyperplasia and neoplasia.

For details about thyroid follicular cell carcinogenesis, the reader should consult appendix C (Hill et al., 1989) and appendix D, an update of the science since 1988 (Hard, 1996); only a limited number of references are in this text. A number of recent papers and reviews are also valuable information sources (Ward and Ohshima, 1986; Paynter et al., 1988; Capen and Martin,

Figure 1

Hypothalamic-Pituitary-Thyroid Axis



1989; Gaitan, 1989; McClain, 1989, 1992, 1995; Wynford-Thomas and Williams, 1989; Curran and De Groot, 1991; Thomas and Williams, 1991; Capen, 1992, 1994; Williams, 1992, 1995; Farid et al., 1994). The long history and extensive database on thyroid neoplasia also are demonstrated in many older reviews (Bielschowsky, 1955; Morris, 1955; Furth, 1969; Doniach, 1970a, b; Christov and Raichev, 1972; Berenblum, 1974; Jull, 1976).

1.1. ROLE OF TSH IN RODENT CARCINOGENESIS

Several experimental findings in rodents that perturb thyroid-pituitary homeostasis and lead to elevated TSH levels indicate the central role of TSH in inducing thyroid carcinogenic effects: (1) loss of thyroid cells through partial thyroidectomy leads to a sustained inability of the thyroid to meet the demands for thyroid hormone, (2) iodide deficiency decreases the thyroid's ability to synthesize adequate supplies of thyroid hormones, and (3) transplantation of pituitary tumors that autonomously secrete TSH adds more of the trophic hormone to graft recipients (Hill et al., 1989). Note that these experimental manipulations are done in the absence of any exogenous chemical treatment but demonstrate the seminal qualitative role that TSH plays in thyroid carcinogenesis. Quantitatively, its significance is demonstrated by the correlation between the TSH level and number of tumors/gland in an initiation-promotion study (McClain, 1989).

If a goitrogenic stimulus that would lead to thyroid tumor formation in rodents is removed early in the process, effects reverse towards normal (Todd, 1986). Likewise, if a goitrogenic stimulus is given (1) in conjunction with adequate amounts of exogenous thyroid hormone (McClain et al., 1988) or (2) after hypophysectomy to remove TSH-secreting cells (Jemec, 1980), then hypertrophy, hyperplasia, and tumors of the thyroid do not develop. It follows from these observations that if TSH levels are chronically elevated, there will be thyroid cell hypertrophy, hyperplasia, and some potential for neoplasia, but under conditions where thyroid-pituitary homeostasis is maintained, the steps leading to tumor formation are not expected to develop, and the chances of tumor development are negligible.

1.2. GENETIC INFLUENCES

Rodent studies indicate an interplay between genetic and nongenetic events in the development of thyroid tumors. Evidence indicates that carcinogenesis often proceeds through a number of operational steps termed initiation, promotion, and progression (Pitot and Dragan, 1991). Initiation seems to be linked to genetic events with the induction of DNA mutations, whereas promotion includes at least nongenetic events that lead to the expansion of a clone of initiated cells via repeated cell division. Progression is associated with the accumulation of cell behaviors (like enzymatic destruction of basement membranes and increased mobility) that allow

cells to invade locally and metastasize distally, probably in part due to still other mutations. Some genetic influences do not result in mutations but in changes in gene expression that can affect the carcinogenic process.

Treatment regimens that produce thyroid tumors in rodents can be conceptualized in regard to initiation and promotion; four examples are cited. It is recognized that these steps do not in themselves describe carcinogenic mechanisms but, instead, present a framework for viewing experimental findings. (1) In two-stage experiments where a mutagenic agent such as radioactive iodide is followed by treatment with a nonmutagenic goitrogen (e.g., a chemical inhibitor of thyroid hormone synthesis), the first agent acts like an initiator, while the second behaves as a promoter (Doniach, 1953). (2) When treatment with a goitrogen alone leads to tumor formation, TSH increases cell division among normal cells, which leads to increases in the overall chance of a spontaneous initiating mutation and then promotes the altered cells that retain responsiveness to TSH; carcinogenesis in these cases would be free of chemically induced mutagenic effects (Owen et al., 1973). (3) Some chemicals appear to have both initiating and promoting activity because they are mutagenic in many test systems and have significant antithyroid activity (e.g., 4,4'-oxydianiline) (Murthy et al., 1985). (4) Still other agents, such as x-irradiation (NRC, 1990) and certain chemicals (e.g., some nitrosamines) (Hiasa et al., 1991), are definitely mutagenic but lack intrinsic goitrogenic activity. These agents can easily initiate the carcinogenic process, but tumor formation would be independent of strong promotional activity from antithyroid effects. TSH still may play a permissive role because these agents can induce cell injury and cell death, which lead to reductions in the output of thyroid hormone and increases in TSH-induced cell division of initiated thyroid cells.

It thus appears that thyroid cancer in experimental animals may be due to either mutagenic influences that lead to DNA changes; to hormone perturbations that lead to growth stimulation, which directly increases the number of thyroid cells and indirectly leads to mutations; or to a combination of the two (table 1). In those cases where increases in cell number are dominant, the inciting agent or procedure may be seen as the carcinogenic stimulus, but the proximate carcinogenic influence is TSH. For those cases with a dominant mutagenic influence, TSH may play an enhancing role in the carcinogenic process.

Table 1. Carcinogenic influences on the rodent thyroid

DNA directed:
X rays
^{131}I
Mutagenic chemicals
Indirect:
Partial thyroidectomy
Transplantation of TSH-secreting pituitary tumors
Iodide deficiency
Chemicals inhibiting uptake of iodide into the thyroid
Chemicals inhibiting thyroid peroxidase
Chemicals inhibiting release of thyroid hormone from the thyroid gland
Chemicals damaging thyroid follicular cells
Chemicals inhibiting conversion of T_4 to T_3
Chemicals increasing hepatic thyroid hormone metabolism and excretion

1.3. POSSIBLE MECHANISTIC STEPS

The precise molecular steps in the carcinogenic process leading to thyroid follicular cell cancer have not been elucidated totally, although significant insights into the problem have been described (Farid et al., 1994; Said et al., 1994). Normal cell division in the thyroid seems to be affected by an interplay among several mitogenic factors, namely, TSH, insulinlike growth factor 1 (IGF-1), insulin, epidermal growth factor (EGF), and possibly fibroblast growth factor (FGF). Still other factors, such as transforming growth factor β (TGFβ), certain interferons, and interleukin 1, may inhibit growth.

TSH communicates with the cell's interior by activating adenylate cyclase to raise levels of cyclic AMP. It also functions through the phosphatidyl-inositol/Ca^{2+} signal transduction cascade that activates phospholipase C. This latter system expresses itself through two pathways: inositol triphosphate, which releases calcium from cellular stores, and 1,2-diacylglycerol, which activates protein kinase C. Signal transduction continues following protein kinase C activation through several steps, including the *ras* protooncogene and various kinases, culminating in the activation of nuclear transcription factor genes (e.g., *c-fos*), which leads to cellular proliferation. The diacylglycerol pathway may account for the fact that the phorbol ester tumor promoters, which increase protein kinase C, also stimulate thyroid cell division.

EGF, insulin, and IGF-1 act through tyrosine kinase receptors. TSH increases EGF binding to its receptor and enhances cell division. IGF-1 and high doses of insulin may influence the TSH receptor. Iodide decreases thyroid cell adenylate cyclase and calcium levels, whereas reduced iodide enhances TSH effectiveness. In sum, the actual control of normal cell division in follicular cells may, in fact, represent some interaction of all these factors and possibly other growth regulatory substances.

Under conditions of thyroid-pituitary imbalance, there is no question that TSH plays a significant role in stimulating DNA synthesis and cell proliferation. However, there is somewhat of a controversy concerning the extent that normal follicular cells can proliferate. One research group claims that cells have limited capacity to respond to the growth-inducing effects of TSH (Wynford-Thomas et al., 1982). In this case, tumor formation would entail mutational steps that free cells from their growth-limiting potential. Another group of investigators thinks that follicular cells are innately heterogeneous, with some of them having stem cell-like proliferation potential while others are more restricted (Studer and Derwahl, 1995). The stem cell-like follicular cells would continue to respond to TSH stimulation and eventually give rise to tumors. It seems possible that both situations might actually apply.

Neoplastic transformation appears to occur in single cells that then expand clonally. Under TSH stimulation, the yield of mutations that may influence transformation increases, even in the absence of an increase in the mutation rate per cell. This is because the repeated cell divisions lead to an increased number of cells at risk for mutation or because rapid cell turnover leaves some spontaneous DNA damage unrepaired.

The precise genetic alterations that accumulate in thyroid follicular cells have not been clearly established in humans or in experimental systems, although mutations involving the *ras* protooncogene, the p53 tumor suppressor gene, and various chromosome aberrations have been reported in the follicular variety of epithelial tumors. These changes in gene expression could lead to uncontrolled cellular growth and allow cells to attain the ability to invade adjacent tissues and metastasize (table 2). For the papillary variety of thyroid epithelial tumors, changes in expression of other factors have been noted, namely, PTC/ret, trk, and met (Farid et al., 1994; Said et al., 1994).

Transformed rodent cells that are stimulated to proliferate under the influence of continuing antithyroid stimulation retain their responsiveness to TSH. Interestingly, human thyroid cancer cells often retain TSH receptors and the ability to respond to TSH, although their receptors dissipate as tumors become more anaplastic. For tumor cells to attain their maximal malignant potential, they need to lose their dependence on TSH. Although interesting

Table 2. Possible molecular events in human thyroid (follicular) carcinogenesis

Thyroid follicular cells	
⇓	TSH, insulin, IGF-1, EGF, FGF
Nodular hyperplasia	
⇓	*ras*, *gsp*, chromosome aberrations of 5, 7, and 12
Follicular adenoma	
⇓	loss of heterozygosity at 3p
Follicular carcinoma	
⇓	p53
Anaplastic carcinoma	

observations concerning growth regulation associated with thyroid carcinogenesis have been made, clearly more work is needed.

1.4. HUMAN THYROID CARCINOGENESIS

Clinically manifest thyroid cancer in humans in the United States is uncommon and largely nonfatal: only about 13,000 new cases occur each year (an incidence rate of approximately 3/100,000 persons) and about 1,000 deaths annually (>90% 5-year survival, which constitutes only about 0.5% of all cancer deaths) (Boring et al., 1994). In contrast to clinically apparent disease, small occult thyroid cancers are noted at autopsy in a small percentage of persons in a number of surveys and up to about 50% in other investigations (Bondeson and Ljungberg, 1984; Mortenson et al., 1955). The incidence in autopsy studies is more like that noted in rats in the National Toxicology Program, where about 1% of control rats are diagnosed with thyroid cancers at 2 years of age. However, this comparison is somewhat misleading. Detailed histologic examinations of human and rodent thyroids are not routinely performed. Histologic tumor criteria differ over time and across reviewers. In addition, thyroid follicular cell cancer most often is diagnosed histologically as papillary in humans and follicular in rodents. The aggressiveness of tumors varies: rodent thyroid neoplasms rarely metastasize; human cancers frequently metastasize. These differences regarding histology, along with the shortcomings of information from descriptive and analytical epidemiologic investigations, help to emphasize the difficulty in comparing human and rodent cancer incidence data.

For years, the only known human thyroid carcinogen was x-irradiation, causing an increase in papillary tumors, with children being about two-fold or more sensitive than adults (table 3) (NRC, 1990; Ron et al., 1989). There was a question as to whether ionizing radiation

from diagnostic or therapeutic use of radioiodine (^{131}I) was carcinogenic in humans (Holm et al., 1988, 1991), although more recently, children exposed to ^{131}I following the Chernobyl reactor accident in the Ukraine have developed thyroid cancer; iodide deficiency is also common in the region and may augment the response to ^{131}I (IAEA, 1996). To date, no chemical has been identified as being carcinogenic to the human thyroid. Most of the human chemical carcinogens appear to be mutagenic and cause tumors in more than one site; some are steroid hormones. Whether mutagenic chemicals might be more carcinogenic to the thyroids of children is not known.

Humans respond as do experimental animals in regard to *short- and mid-term* disturbances in thyroid functioning from various antithyroid stimuli such as iodide deficiency, partial thyroidectomy (surgically or ^{131}I induced), and goitrogenic chemicals (e.g., thionamides): when circulating thyroid hormone levels go down, the TSH level rises and induces thyroid hypertrophy and hyperplasia.

However, the *long-term* consequences of antithyroid action are harder to interpret, and controversy exists regarding whether the enlarged human thyroid gland undergoes conversion to cancer. Thyroid enlargements and nodules have been implicated as possible antecedents to thyroid cancer in humans, but direct evidence of conversion of these lesions to malignancy is lacking (table 3). Three examples of indirect effects follow. (1) Persons who live in iodide-deficient areas of the world are unable to synthesize adequate levels of thyroid hormones; they develop elevated TSH levels, very enlarged thyroid glands, and lesions typified as adenomatous hyperplasia. There is conflicting evidence whether thyroid cancer is increased in these people (Galanti et al., 1995; Waterhouse et al., 1982). In contrast to these observations, it seems that domestic animals but not wild animals in iodide-deficient areas developed elevated incidences of thyroid tumors (Wegelin, 1928), and tumor incidence disappeared in dogs following the advent of using iodized salt (Ivy, 1947). Iodide may have some influence as to the histologic type of thyroid cancer, with follicular being more common in iodide-deficient areas and papillary being more common in iodide-rich areas (Williams, 1985). (2) People with various inborn errors of metabolism who are unable to synthesize enough effective thyroid hormone develop very enlarged thyroids, but few cases of cancer have been reported. There are no reports of thyroid tumors among persons with resistance to thyroid hormone (Vickery, 1981; Refetoff et al., 1993). (3) In persons with the autoimmune disorder Graves' disease, there are often immunoglobulins that stimulate thyroid cells in ways analogous to TSH, even though TSH levels per se are very low. It is not settled as to whether there is also an increase in thyroid cancer among these patients; some studies seem to indicate either that cancer incidence may be increased or that

Table 3. Carcinogenic influences on the human thyroid

DNA directed: X-rays ^{131}I
Indirect factors may include Iodide deficiency Inborn errors of thyroid hormone metabolism Graves' disease

thyroid tumors in these patients may be more aggressive (Belfiore et al., 1990; Mazzaferri, 1990). Overall, this qualitative information suggests that prolonged stimulation of the human thyroid under certain circumstances may lead to cancer, as in the presence of inherited metabolic conditions or long-term immunologic abnormalities, but there is uncertainty in this conclusion.

In epidemiologic studies, goiter and thyroid nodules have been shown to be risk factors for thyroid cancer. The specific causes of these enlargements are not known but, where studied, do not appear to be due to hypothyroidism (McTiernan et al., 1984; Ron et al., 1987). Some researchers believe that part of the association may be due to the close medical scrutiny given to persons with suspicious thyroid enlargements (Ridgway, 1992; Mazzaferri, 1993).

In spite of the potential qualitative similarities, there is evidence that humans may not be as sensitive quantitatively to thyroid cancer development from thyroid-pituitary disruption as rodents. Rodents readily respond to reduced iodide intake with the development of cancer; humans develop profound hyperplasia with "adenomatous" changes with only suggestive evidence of malignancy. Even with congenital goiters due to inherited blocks in thyroid hormone production, only a few malignancies have been found in humans.

The reasons for differences in perceived interspecies sensitivity are not really known. However, one factor that may play a role in interspecies quantitative sensitivity to thyroid stimulation deals with the influence of protein carriers of thyroid hormones in the blood (table 4). Both humans and rodents have nonspecific low-affinity protein carriers of thyroid hormones (e.g., albumin). However, in humans, other primates, and dogs there is a high-affinity binding protein, thyroxine-binding globulin, which binds T_4 (and T_3 to a lesser degree); this protein is missing in rodents and lower vertebrates. As a result, more T_4 remains bound to proteins with lower affinity in the rodent and is more susceptible to removal from the blood, metabolism, and excretion from the body. In keeping with this finding, the serum half-life of T_4 is much shorter in rats (less than 1 day) than in humans (5 to 9 days); this difference in T_4 half-life results in a 10-fold greater requirement for exogenous T_4 in the rat with a nonfunctioning thyroid than in the

2. SCIENCE POLICY GUIDANCE

2.1. SCIENCE POLICY STATEMENTS

Rodents and humans share a common physiology in regard to the thyroid-pituitary feedback system. Short-term perturbation in this system often leads to similar effects in both species resulting in increases and decreases in circulating thyroid and pituitary hormones. It is well established in rodents that disruption of thyroid-pituitary status with elevation of TSH levels is associated with thyroid tumor and sometimes related pituitary tumor development. This is true whether it is due to deficiency in iodide, reduction in thyroid mass, presence of TSH-producing pituitary tumors, or administration of goitrogenic chemicals. An increase in TSH stimulation of the thyroid is a final common pathway. Likewise, administration of exogenous thyroid hormone or removal of a TSH-increasing stimulus reduces the effects in the thyroid.

The role of thyroid-pituitary disruption in cancer development in humans is much less convincing than in animals. Iodide deficiency is associated with increases in thyroid cancer in some studies but not others. Similarly, an association between either inborn errors of metabolism affecting thyroid hormone output or autoimmune-related Graves' disease and cancer is suggested but not proved. It seems that TSH may at least play some permissive role in carcinogenesis in humans. Accordingly, one cannot *qualitatively* reject the animal model; it seems reasonable that it may serve as an indicator of a potential human thyroid cancer hazard. However, to the extent that humans are susceptible to the tumor-inducing effects of thyroid-pituitary disruption and given that definitive human data are not available, it would appear that *quantitatively* humans are less sensitive than rodents in regard to developing cancer from perturbations in thyroid-pituitary status.

Rodents develop thyroid cancer from ionizing radiation, working by a mutagenic mode of action. Ionizing radiation is the only verified human thyroid carcinogen, and children are more sensitive than adults. The role of mutagenic chemicals in human thyroid carcinogenesis and in children is unknown. Recognizing mode of action information linking thyroid-pituitary disruption and mutagenesis to thyroid carcinogenesis, the Agency adopts the following science policy:

1. It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid.
2. In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. This is a conservative position when thyroid-pituitary disruption is the sole mode of action,

because rodents appear to be more sensitive to this carcinogenic mode of action than humans. When the thyroid carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than adults needs to be evaluated on a case by case basis.

3. Adverse rodent noncancer thyroid effects (e.g., thyroid gland enlargements) following short- and long-term reductions in thyroid hormone levels are presumed to pose human noncancer health hazards.

Some chemicals that have produced thyroid follicular cell tumors in laboratory rodents appear to work by producing a derangement in thyroid-pituitary homeostasis; others appear to act primarily through a mutagenic mode of action; and still others seem to show a combination of both modes of action. The question then becomes how to evaluate the risks of thyroid tumors for humans given exposure to any of these chemicals. If the animal tumors are due to chemical doses that produce imbalances in thyroid-pituitary functioning, it is anticipated that the chance of cancer is minimal under conditions of hormonal homeostasis. Tumors seeming to arise from relevant mutagenic influences (e.g., gene mutations and structural chromosome aberrations) without perturbation in thyroid-pituitary status may pose some chance of cancer across a broader range of doses. Consequently, until such time that biologically based models and data become available, EPA adopts the following science policy for conducting dose-response assessments of chemical substances that have produced thyroid follicular cell (and related pituitary) tumors in experimental animals:

1. A linear dose-response procedure should be assumed when needed experimental data to understand the cause of thyroid tumors are absent and the mode of action is unknown (table 5, example 1) (See case study for compound 2 in appendix A).
2. A linear dose-response procedure should be assumed when the mode of action underlying thyroid tumors is judged to involve mutagenicity alone.
3. A margin of exposure dose-response procedure based on nonlinearity of effects should be used when thyroid-pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors (table 5, example 3) (See case study for compound 1 in appendix A). Thyroid-pituitary perturbation is not likely to have carcinogenic potential in short-term or highly infrequent exposure conditions. The margin of exposure procedure generally should be based on thyroid-pituitary disruptive effects themselves, in lieu of tumor effects, when data permit. Such analyses will aid in the development of combined noncancer and cancer

Table 4. Inter- and intraspecies differences

Parameter	Human	Rat
Thyroxine-binding globulin	present	essentially absent
T_4 Half-life	5-9 days	0.5-1 day
T_3 Half-life	1 day	0.25 day
T_4 Production rate/kg b.w.	1 ×	10 × that in humans
TSH	1 ×	6-60 × that in humans
Follicular cell morphology	low cuboidal	cuboidal
Sex differences		
Serum TSH	sexes equal	$M \leq 2 \times F$ [a]
Cancer sensitivity	$F = 2.5 \times M$	$M > F$

[a]M = male; F = female.

adult human (Döhler et al., 1979). Serum T_3 levels also show a species difference; the half-life in rats is about 6 hr while that in humans is about 24 hr (Oppenheimer, 1979; Larsen, 1982). There is a morphological consequence to these hormone differences. High thyroid hormone synthetic activity is demonstrated in follicles in rodents: they are relatively small, surrounded often by cuboidal epithelium. Follicles in primates demonstrate less activity and are large with abundant colloid, and follicular cells are relatively flattened (low cuboidal) (McClain, 1992).

The accelerated production of thyroid hormones in the rat is driven by serum TSH levels that are probably about 6- to 60-fold higher than in humans. This assumes a basal TSH level in rats and humans of 200 ng/ml and 5 μU/ml, respectively, and a potency of human TSH of 1.5 to 15 U/mg of hormone (NIDDK, 1994). Thus, it appears that the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased turnover of thyroid hormones. It follows that increases in TSH levels above basal levels in rats could more readily move the gland towards increased growth and potential neoplastic change than in humans. Interestingly, adult male rats have higher serum TSH levels than females (Chen, 1984), and they are often more sensitive to goitrogenic stimulation and thyroid carcinogenesis. In humans, there is no sex difference in hormone levels, but females more frequently develop thyroid cancer (Boring et al., 1994).

In addition to considerations about the influence of serum thyroid hormone carrier proteins, there are differences between humans and animals in size, lifespan, and pharmacokinetics and pharmacodynamics of endogenous and exogenous chemicals. Any comparison of thyroid carcinogenic responses across species should be cognizant of all these factors.

The guidance given here on thyroid tumors is not unique. Other authorities have recognized and incorporated advances in the understanding about carcinogenic mechanisms into their assessments of cancer risks (JMPR, 1990; IARC, 1991; JECFA, 1991; Vainio et al., 1992; Poulsen, 1993; Strauss et al., 1994).

assessments of toxicity. Results of the margin of exposure procedure will be presented in a way that supports risk management decisions for exposure scenarios of differing types (e.g., infrequent exposure, short durations).

4. Consistent with EPA risk characterization principles, both linear and margin of exposure considerations should be assumed when both mutagenic and thyroid-pituitary disruption modes of action are judged to be potentially at work (table 5, example 4) (See case study for compound 3 in appendix A). The weight of evidence for emphasizing one over the other should also be presented. The applicability of each to different exposure scenarios should be developed for risk management consideration.
5. Dose-response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mode of action information bearing on their induction and principles laid out in current EPA cancer risk assessment guidelines. There is an association between thyroid and liver tumors in rodent cancer studies (McConnell, 1992; Haseman and Lockhart, 1993). The reason(s) for this relationship has not been generically established but should be carefully assessed for chemicals on a case-by-case basis. Some may be due to induction of hepatic microsomal enzymes.

Most of the focus in implementing this policy is devoted to answering the following questions: (1) Does an agent that shows thyroid carcinogenic effects have antithyroid activity? (2) Can modes of action other than thyroid-pituitary disruption account for thyroid tumor formation by this chemical?, and (3) How can one express thyroid dose-response relationships? Adequately answering these questions is dependent on a *data-rich* information base for the chemical under review. To the extent practicable, an effort should be made to review such information before deciding on the possible mode of chemical action underlying the thyroid tumors and their consequences for risk assessment. The procedures and considerations developed in this report embody current scientific knowledge of thyroid carcinogenesis and evolving science policy. Should significant new information become available, the Agency will update its guidance accordingly.

Table 5. Default dose-response procedures for thyroid carcinogens

Example	Array of effects		Dose-response methodology
	Mutagenic	Antithyroid	
1	either or both unknown		linear
2	yes	no	linear
3	no	yes	margin of exposure
4	yes	yes	linear and margin of exposure

2.2. EVIDENCE FOR ANTITHYROID ACTIVITY

Different types of information on a chemical may be obtainable indicating that it has antithyroid activity, that is, whether it works via disruption of thyroid-pituitary status. These include effects manifest in the thyroid gland per se, various tissues peripheral to the thyroid, and the liver. All available factors are assembled into an overall evaluation of the likelihood the chemical is acting via disruption of the thyroid-pituitary axis.

2.2.1. Data Needs

Special mechanistic studies are needed to demonstrate chemically induced perturbations in thyroid-pituitary functioning. Repeat dose (e.g., 2 to 4 week and 13 week) studies that have simultaneously evaluated a number of endpoints (as discussed below) often can provide critical information for evaluating qualitatively whether antithyroid activity exists and what it is due to and quantitatively what dose-response relationships may pertain. Such studies should be designed carefully to encompass *multiple doses* ranging from above those clearly associated with tumors in chronic studies down to those below which there is no indication of disturbance in critical thyroid-pituitary parameters, so that *dose-response* relationships can be defined. Special attention should be given to the time of sampling of thyroid and pituitary hormones because of the compensatory action of homeostatic mechanisms and the difficulty in discerning changes after compensation occurs. Hormone sampling also should be conducted at the same time during the course of a day, and efforts should minimize stress in handling animals. Effects measured only at the end of chronic rodent studies are often difficult to evaluate and, alone, seldom provide adequate information.

Of the eight listed areas of inquiry shown in table 6, the determination of the antithyroid activity of a chemical *requires* empirical demonstration of the following five items. Demonstration of (1) increases in thyroid growth and (2) changes in thyroid and pituitary hormones are

considered to be the most important. (3) Location of the site(s) of antithyroid action documents where in the body the chemical under assessment leads to perturbations in

Table 6. Data demonstrating antithyroid activity

Required	Desirable
1. Increases in cellular growth	6. Lesion progression
2. Hormone changes	7. Structure-activity relationships
3. Site of action	8. Other studies
4. Dose correlations	
5. Reversibility	

thyroid-pituitary functioning. Next is the demonstration of (4) dose correlations among various effects so as to determine where the growth curve for the thyroid gland deviates from the normal pattern of cell replacement and how this relates to doses producing tumors. (5) Reversibility of effects following treatment cessation during the early stages of disruption of the thyroid-pituitary axis shows that permanent, self-perpetuating processes have not been set into motion. The remaining three listed items are *desirable*: (6) lesion progression, (7) structure-activity analysis, and (8) other studies. Each provides supporting information that can add profoundly to the assessment of an agent's ability to produce antithyroid effects.

2.2.2. Increases in Cellular Growth (evidence required)

Agents that affect thyroid-pituitary functioning stimulate thyroid enlargement. Commonly measured parameters include but are not limited to increases in absolute or relative thyroid gland weight or to histologic indications of cellular hypertrophy and hyperplasia, morphometric documentation of alteration in thyroid cellular components, and changes in the proliferation of follicular cells detected by DNA labeling or mitotic indices.

2.2.3. Hormone Changes (evidence required)

With a disruption in thyroid-pituitary functioning, there is typically a reduction in both circulating serum T_4 and T_3 concentrations and an increase in TSH levels within days or a few weeks of chemical administration. In some cases, T_4 levels may be lowered while T_3 levels are maintained within normal limits. In addition, sometimes hormone levels may return to normal over time for mild goitrogenic agents because of the homeostatic compensatory increase in

thyroid activity and mass. Statistical tests can help evaluate the significance of hormone perturbations, but it is the constellation of changes in both thyroid and pituitary hormones that indicate whether the negative feedback loop between the thyroid and pituitary has been perturbed.

2.2.4. Site of Action (evidence required)

Chemicals that produce thyroid tumors alone or after administration of a mutagenic initiator produce interference with thyroid-pituitary function by a variety of specific means (figure 2; also see appendix B). Effects have been found at one or more of the following anatomical locations: intrathyroidal and various extrathyroidal sites, including the liver and possible other sites. Sometimes clues as to the site of action can be deduced by analysis of structurally related compounds (also see section 2.2.7). Generally, enough information on a chemical should be given to be able to identify the sites that contribute the major effect on thyroid-pituitary function. Given experience to date, it appears that most often the liver is the site of action, followed by the thyroid, where thyroid peroxidase is affected; other sites of action seem to be less common.

2.2.4.1. *Intrathyroidal*

Several different effects in the thyroid gland have been associated with the development of antithyroid activity and the formation of thyroid tumors in rodents. *Iodide pump inhibition* by chemicals like thiocyanate and perchlorate ions leads to a decrease in uptake of inorganic iodide into the thyroid gland. *Thyroid peroxidase inhibition* blocks the incorporation of active iodide into iodotyrosines and their coupling to form the nascent thyroid hormones. Agents that are known to reversibly or irreversibly inactivate this enzyme include various thionamides such as 6-propylthiouracil and ethylene thiourea, certain aromatic amines such as some of the sulfonamides, and miscellaneous compounds such as amitrole. *Toxicity to thyroid cells*, as has been seen with polychlorinated biphenyls, may affect the gland's ability to manufacture and secrete thyroid hormones. *Inhibition of thyroid hormone release*, with agents such as lithium and excess iodide, results in a retention of hormones within the colloid and a paucity released into the circulation (Green, 1978).

2.2.4.2. *Extrathyroidal*

2.2.4.2.1. ***Peripheral tissues.*** Several tissues and organs of the body, including skeletal muscle, kidneys, and liver, contain different deiodinases that remove iodine atoms from thyroid hormones. *Inhibition of 5'-monodeiodinase*, the enzyme that normally converts T_4 to T_3, leads to a reduction in circulating T_3 and an increase in the rT_3 level via 3'-deiodination. Compounds such as FD&C Red No. 3 (erythrosine), iopanoic acid, and 6-propylthiouracil act by competitive inhibition of this enzyme or interaction with its sulfhydryl cofactor. The deiodinase system in the pituitary is somewhat different from that in the periphery and may respond differently to certain chemicals (Chanoine et al., 1993).

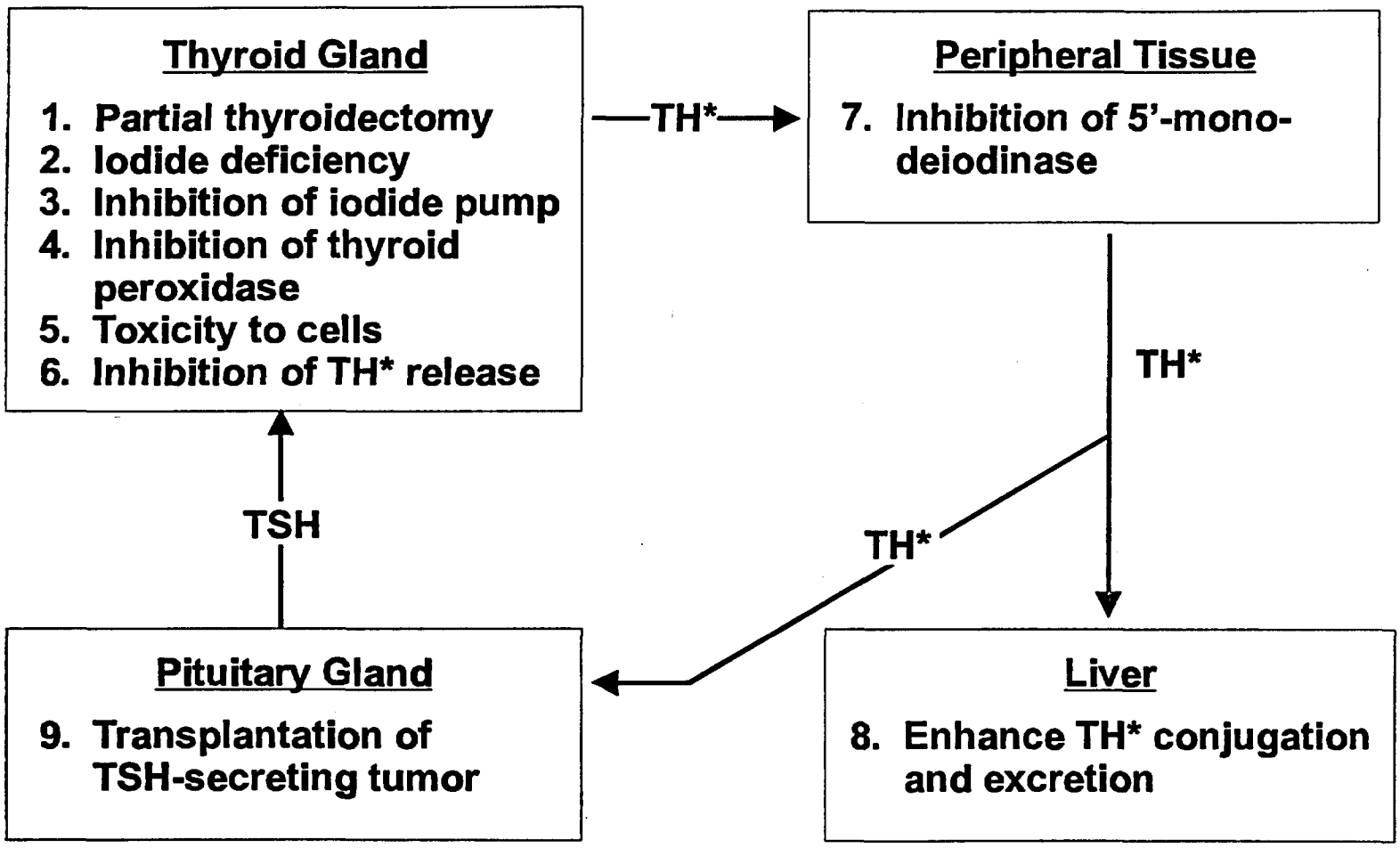
2.2.4.2.2. ***Liver.*** A significant amount of thyroid hormone normally is metabolized by the liver. Certain chemicals induce liver microsomal enzymes and enhance thyroid hormone metabolism and removal. T_4 conjugation with glucuronic acid is enhanced by those agents that induce hepatic glucuronosyl transferase (Curran and De Groot, 1991). In these cases, thyroid hormone also may show increased binding to hepatocytes, increased biliary excretion, and increased plasma clearance. Other common manifestations of microsomal induction include such things as enlargement of hepatocytes in the centrolobular region, increase in hepatic cell smooth endoplasmic reticulum, increase in P-450-associated metabolism of various chemical substrates, and increase in biliary flow.

Disparate chemical and functional classes such as polyhalogenated hydrocarbons (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]) and barbiturates (e.g., phenobarbital) as well as various individual compounds (e.g., the pesticide clofentezine and the drugs spironolactone and the histamine [H2] antagonist SK&F 934790) are known to enhance thyroid hormone excretion via effects on microsomal enzymes. Conjugation also may occur with sulfate, usually associated with deiodination; deamination, oxidative decarboxylation, and ether-link cleavage are minor degradative pathways. Interestingly, phenobarbital has been shown to be a promoter in the rodent thyroid (McClain et al., 1988), but there is no indication it produces thyroid cancer in humans (Olsen et al., 1989).

2.2.4.2.3. ***Other potential sites.*** Chemicals might *bind thyroid hormone receptors* and produce certain effects. For instance, agents (e.g., salicylates) could displace thyroid hormone from plasma carrier proteins and result in reductions in effective thyroid hormone (Oppenheimer and Tavernetti, 1962; Hershman, 1963). They could bind receptors in target organs (e.g., pituitary) but result in inactive complexes. These possibilities as well as other potential sites of action

Figure 2

Antithyroid Effects Influencing Thyroid Carcinogenesis



(e.g., affecting thyroid-releasing hormone, thyroid hormone-responsive elements in the DNA) might be conceived as influencing thyroid-pituitary functioning.

2.2.5. Dose Correlations (evidence required)

Confidence in an antithyroid mode of action is enhanced by evidence of a correlation between doses of a chemical that do and do not jointly perturb thyroid-pituitary hormone levels, produce various histologic changes in the thyroid, and/or produce other effects, including thyroid cancer. These are important steps in evaluating the significance of thyroid-pituitary disruption in thyroid carcinogenesis and in evaluating dose-response relationships (see section 2.4).

2.2.6. Reversibility (evidence required)

Chemicals working through an antithyroid mode of action induce changes in thyroid cell morphology and number and in thyroid-pituitary hormones that are reversible upon cessation of chemical dosing.

2.2.7. Lesion Progression (evidence desirable)

Evidence for a progression of histologic lesions over time following exposure to an agent, including cellular hypertrophy and hyperplasia, focal hyperplasia, and neoplasia (benign and possibly malignant tumors), is commonly noted.

2.2.8. Structure-Activity Analysis (evidence desirable)

Analysis of chemical structure may show that an agent belongs to a class of compounds that induces thyroid tumors via thyroid-pituitary imbalance (e.g., agents that inhibit thyroid peroxidase, liver microsomal enzyme inducers) (see appendix B). This allows for the scientific inference that the chemical under review may act similarly. In addition, generic information developed on a group of analogues can be used to support the assessment of the agent under review.

2.2.9. Other Studies (evidence desirable)

Many other studies bearing on thyroid-pituitary imbalance can provide a range of findings from strong ancillary information to only suggestive indications. A few of these studies are noted here, for example, suppression of induced effects by concurrent administration of thyroid hormone; absence of initiating activity but presence of promoting activity in two-stage carcinogenicity tests; localization of certain chemicals in the thyroid (e.g., thionamides); influences on hypothalamic responsiveness to thyroid hormone levels and output of thyrotropin- releasing

factor; changes in TSH mRNA transcripts in the pituitary; and alteration of thyroid hormone nuclear receptor number or synthesis.

2.3. OTHER MODES OF CARCINOGENIC ACTION

Another critical element in the evaluation of a thyroid carcinogen is a determination of whether mutagenicity may account for the observed tumors. Primary emphasis should be placed on those endpoints that have mechanistic relevance to carcinogenicity. DNA reactivity is a prime predictor of potential mutagenic carcinogenicity. Many of these compounds belong to particular chemical classes (e.g., aromatic amines, nitrosamines, polyaromatic hydrocarbons). These chemicals or their metabolites bind to DNA, and they often induce gene mutations and structural chromosome aberrations. In recognition that organs and tissues may have unique metabolic activity, it is helpful to know in addition to traditional short-term test results whether there is evidence of DNA reactivity in target tissues (e.g., DNA adducts, unscheduled DNA synthesis, single-strand breaks).

Mutagenic effects other than those associated with direct DNA reactivity need to be carefully evaluated in regard to their mechanistic implications; some may have different cancer dose-response considerations than do directly DNA reactive agents. Included here are items such as the ability of the chemical under review to induce indirect effects on DNA, such as through influence on the cell division spindle or production of reactive oxygen. Agents also should be evaluated for the presence of structural alerts that are often predictive of chemical reactivity or potential carcinogenicity. All these findings are then melded into an overall appraisal of an agent's ability to influence genetic processes relevant to carcinogenesis in the thyroid or other sites.

It is possible that information on carcinogenic modes of action other than mutagenicity or thyroid-pituitary derangement will become available. If so, this information also needs to be incorporated on a case-by-case basis into the evaluation of a chemical's ability to produce tumors of the thyroid (and of other sites).

2.4. DOSE-RESPONSE CONSIDERATIONS

Evaluation of potential dose-response relationships for thyroid tumors depends on an evaluation of the chemical's expected mode of carcinogenic action. Major determinations include whether the thyroid tumors appear to be due at least in part to thyroid-pituitary imbalance and whether other modes of action (e.g., relevant mutagenicity) may be pertinent to their formation. Other case-specific factors may provide crucial information, such as the extent to which data gaps and uncertainties prevail. A *rationale* should accompany the selection of any dose-response

method. Guidance is provided below in the subparts of this section, and the case studies presented in appendix A help to illustrate ways one might evaluate data and make judgments about potential thyroid cancer dose-response relationships.

When tumor types other than thyroid follicular cell (and related pituitary) pertain, mode-of-action and other considerations should help guide the selection of the appropriate dose-response extrapolation method(s). Separate dose-response extrapolations may apply for the different tumor types, depending on the specifics of the case.

2.4.1. Thyroid Tumor High-to-Low Dose Extrapolation

If antithyroid influences are operative in the formation of thyroid tumors, attention should be directed to biologically based procedures that embody the mechanistic influences, if they are available. In their absence, default procedures should be employed that incorporate nonlinear or linear considerations. When thyroid-pituitary imbalance is not operative, other mode of action or default considerations should be utilized; generally low-dose linear extrapolations are appropriate. All extrapolation procedures should be consistent with the guidance given in the EPA cancer risk assessment guidelines (U.S. EPA, 1986a, 1996). Finally, when thyroid tumors seem to arise from both a chemical's antithyroid activity and its mutagenic potential, dose-response relationships might be projected in ways that express concerns for both possible modes of action.

2.4.1.1. ***Biologically Based.*** Optimally, mechanistic considerations that underlie thyroid tumor formation would be incorporated into biologically based extrapolation models. They should include physiologically based pharmacokinetic considerations for the chemical and its interactions with and effects on cells. The trouble is that generic biologically based dose-response extrapolation models have yet to be developed and validated for the thyroid. Fortunately, work in this area is commencing, and a model has been developed to explain effects associated with TCDD (Kohn et al., 1996). Until mechanistic models become generally available and chemical-specific data have been produced, the Agency assessments will employ, as discussed below, *one of three default* procedures in its evaluation of thyroid cancer risks: nonlinear, linear, or both nonlinear and linear.

2.4.1.2. ***Nonlinear.*** For those cases where thyroid tumors arise from chemically induced disturbances in thyroid-pituitary functioning, tumors are considered to be secondary to the adverse effects on thyroid gland function that precede them. As exposures to such agents decrease, the likelihood of cancer decreases; risks may be seen as minimal at doses where there is no effect on thyroid-pituitary homeostasis. Generally, homeostasis is considered to apply when

serum T_4, T_3, and TSH levels and thyroid and pituitary morphology and growth are within their normal limits (see case study for compound 1 in appendix A). Risk assessments on agents should contain case-specific and generic information that support the contention that nonlinear dose-response relationships apply.

Empirically, there is some support for thyroid follicular cell tumors having a dose-response curve that is less than linear (curvilinear upward). Using the Weibull model to give some indication of the shape of the dose-response curve and the rodent tumor incidence database of the National Cancer Institute/National Toxicology Program, slope functions were calculated. Incidence for tumors in general was more consistent with a quadratic than a linear dose-response curve shape (Meier et al., 1993; Hoel and Portier, 1994). However, when slopes for thyroid follicular cell tumors were compared with those of thyroid C cell tumors and all other tumor sites, those from the thyroid follicular cells were even more curvilinear than the others (Portier, personal communication, September 8, 1994).

One argument for assuming low-dose linearity in dose-response assessments is the concept of additivity to background. If a given chemical acts in the same way or augments an endogenous or exogenous background factor that contributes to tumor development, then the effect of the chemical will add to that of the background factor. The result is low-dose linearity up to at least a doubling of the background rate (Crump et al., 1976). The concept may not be applicable to certain processes subject to hormonal regulation, such as with the thyroid gland. Normally the level of circulating thyroid hormone is adjusted carefully by the negative feedback with the pituitary. Elevations and reductions in thyroid hormone are met with adjustments in the amount of TSH released from the pituitary so as to bring thyroid hormone values back into the normal range. These excursions are not part of an ongoing carcinogenic process; instead, they represent the body's means of maintaining thyroid hormone homeostasis. Small doses of a potentially antithyroid chemical may not result in any perturbation in hormone levels or stimulation of thyroid follicular cell growth simply because homeostatic mechanisms will drive thyroid hormone levels back into the normal range.

The Agency acknowledges that it may be difficult to establish a precise dose where there is negligible response for a specific toxicological effect given the sensitivity of methods to evaluate various parameters and the variability in measurement of endpoints. In recognition of this, it is incumbent on the scientific community to help in the transfer of various molecular techniques from the research laboratory to testing facilities (e.g., measurement of hormone receptor mRNA production and receptor content, occupancy, and turnover) that may be more sensitive indicators of thyroid-pituitary malfunction.

The way the Agency has dealt with nonlinear phenomena is to express concern for human exposure ("risk") as a *margin of exposure* (MOE), the ratio between a dose point of departure for

the critical effect and the relevant estimate of anticipated human exposure (incorporating dose, frequency, and time). Large MOEs are attended with less concern than are small ones. Traditionally, the point of departure is expressed as a no-observed-adverse-effect level (NOAEL), and the critical effect is the relevant toxicological endpoint occurring at the lowest doses in a toxicological study (see sections 2.4.2 and 2.4.3). More recently, alternatives to the NOAEL have been proposed to serve as points of departure for MOE calculation.[1] Procedures should be employed for thyroid tumors that are consistent with EPA cancer risk assessment guidelines and practices that are applicable at the time.

Assessments should include adequate information to aid in interpreting the significance of MOEs, such as taking into consideration the variability in sensitivity among individuals within a species, the sensitivity of humans relative to experimental animals, and other strengths, weaknesses, and uncertainties that are part of the assessment. Decision makers must then judge the adequacy of the MOE for their risk management purposes.

2.4.1.3. ***Low-Dose Linear.*** For those assessment cases where thyroid tumors do not seem to be due to thyroid-pituitary imbalance, existing case-specific mode of action information and default considerations should be used to develop dose-response relationships. In other cases, there may be an absence of mode of action information for an agent. Generally, a low-dose linear default for the thyroid tumors may be contemplated in these two circumstances in accordance with current EPA procedures (see case studies for compounds 2 and 4 in appendix A). Recent cancer risk assessment guideline proposals suggest that linear extrapolation would involve calculation of a dose point of departure with a straight line extrapolation from there to the origin.[1]

2.4.1.4. ***Low-Dose Linear and Nonlinear.*** Finally, careful review is warranted when both antithyroid and other determinants seem to apply to the observed thyroid tumors, such as when there are certain mutagenic influences (e.g., structural chromosome aberrations). Judgments with accompanying scientific reasoning should be presented on the most appropriate way(s) to evaluate

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[1]In April 1996, EPA proposed a revision of its existing cancer risk assessment guidelines (U.S. EPA, 1986a). A dose point of departure is determined by extrapolating effects in the observed part of the dose-response curve. It is used, depending on the expected mode(s) of action, as the starting point for either linear extrapolation to the origin or calculation of an MOE in the case of nonlinear extrapolation. Generally, tumor or nontumor (e.g., hyperplasia) endpoint incidence is extrapolated to the 10% effect level. The lower 95% confidence limit on that dose may be used as the point of departure. Possibly, the point estimate at the 10% effect level may be used in lieu of the lower-bound estimate given in the proposal. Other means of determining departure points are also proposed (U.S. EPA, 1996). The final cancer guidelines will clarify these matters.

thyroid risk: either linear or nonlinear or, when the two procedures are about equally tenable, both. When both procedures are presented, assessors should state the relative merits of each procedure. In some cases, one of the two methods may be preferable and should be given more weight; the rationale for conclusions should be expressly presented. Projected risks using linear extrapolation often give rise to concerns at doses lower than those when nonlinear techniques are applied. Thus, these two techniques usually can be seen as putting lower and upper bounds on exposures of concern (see case study for compound 3 in appendix A). Assessments should include guidance for decision makers in interpreting concerns for exposure when both extrapolation techniques are presented.

2.4.2. Endpoints to be Employed

One optimally would have access to data on various preneoplastic endpoints that would be evaluated (following short-term [e.g., 2 to 4 week] and subchronic [e.g., 13 week] studies) and compared with the doses that have produced tumors in chronic studies. Endpoints that should regularly be evaluated and presented in dose-response analyses include (1) changes in levels of T_4 and T_3, (2) increases in TSH, (3) the incidence of thyroid follicular cell hypertrophy, hyperplasia, and neoplasia, (4) increases in cell proliferation and thyroid weight, and (5) specific endpoints associated with thyroid-pituitary disturbance at the site(s) of chemical action (e.g., inhibition of thyroid peroxidase, increased metabolism, and clearance of thyroid hormone). A host of other effects as discussed above could be monitored in the thyroid, pituitary, or thyroid hormone-responsive organs and included on a case-by-case basis. Care needs to be taken to ensure that studies to evaluate these parameters have been conducted (1) for adequate periods of time and (2) at doses that clearly define dose-response relationships. Attention also needs to be given to procedures that help reduce the variability in responses among animals (e.g., time and means of animal sacrifice and tissue sampling).

2.4.3. Estimation of Points of Departure

For the important toxicity studies, a point of departure (e.g., NOAEL) is determined for each thyroid toxicity endpoint and exposure duration. Doses associated with tumors also should be noted. The departure point may be a study dose or an estimated dose. For instance, when data permit, the departure point can be estimated by extrapolation of doses associated with observed responses (e.g., TSH levels) to those attended with no significant deviation from the control range (see case study for compound 1 in appendix A). In other cases, an appropriate observed study NOAEL may be selected (see case study for compound 3 in appendix A) or other procedures in accordance with EPA guidance may be used.

Considerations for the selection of the critical endpoint to be used to project thyroid cancer risk (i.e., calculation of the MOE) include (1) the nature of the endpoint and its relationship to the perturbations in endocrine balance and carcinogenicity, (2) the presence of good dose-response or dose-severity of effect relationships, (3) the sensitivity of the endpoint vis-à-vis other potential endpoints, and (4) the length of the dosing period and its relevance to making judgments about the consequences of potential chronic exposures.

2.4.4. Interspecies Extrapolation

Many considerations are relevant in attempting to extrapolate thyroid carcinogenic effects in experimental animals to humans. The relative sensitivity of humans and rodents to the carcinogenic effects of elevated TSH are not firmly established, but important observations have been made. Given that the rodent is a sensitive model for measuring the carcinogenic influences of TSH and that humans appear to be less responsive (as developed in section 1.4), one would expect that projections of potential risk for rodents would serve as conservative potential indicators of risks for humans.

Rodent cancer studies typically include doses that lead to toxicity, including perturbation in thyroid-pituitary functioning, over a lifetime. The relevance of such experimental conditions to anticipated human exposure scenarios (i.e., dose, frequency, and time) should be considered and presented in the final characterization of risk. This is especially true because thyroidal effects are not necessarily expected at all doses. In addition, chemically induced effects that are produced by short-term disruption in thyroid-pituitary functioning appear to be reversible when the stimulus is removed.

Although it appears that humans are less sensitive to the carcinogenic perturbations of thyroid-pituitary status than rodents (e.g., iodide deficiency), such determinations should be made on a case-by-case basis. This would depend on a host of factors involving the agent, including the depth and breadth of the database, the congruence of the information supporting a given mode of action, and the existence of information on humans. Decision makers should be apprised of risk assessment judgments and their rationales. In the absence of chemical-specific information, the default assumption is that humans should be considered to be as sensitive to carcinogenic effects as rodents. That is to say, a factor of one would be used when extrapolating effects in rodents to those in humans.

2.4.5. Human Intraspecies Evaluations

Thyroid hormones are regulated within rather narrow ranges, with normal adult human serum values often being given as T4--4 to 11 ug/dL and T3--80 to 180 ng/dL. TSH levels

extend over a broader range--0.4 to 8 uU/ml, due to the incorporation in recent years of more sensitive laboratory methods that have extended the normal range to lower values (Ingbar & Woeber, 1981; Surks et al., 1990). The upper bound on normal TSH has not changed, and it is the one of import to considerations of antithyroid effects of chemicals. During development somewhat higher levels for each of the hormones are noted, with adult hormone values being reached beyond about 10 years of age (Nicholson and Pesce, 1992). Growth of the thyroid gland continues for the first 15 years of life, going from about 1 gram at birth to an adult size of about 17 grams (Fisher and Klein, 1981; Larsen, 1982). The control of normal thyroid growth during development is not totally known, although the increase in gland size may be independent of TSH stimulation (Logothetopoulus, 1963).

Extended deviations in human thyroid hormone levels either above or below the normal range are associated with the disease states, hyperthyroidism and hypothyroidism, respectively. Worldwide, iodide deficiency is the most prominent cause of thyroid disease generally, and hypothyroidism specifically. In these areas, children quickly manifest characteristic symptoms and signs which persist throughout life (Bachtarzi and Benmiloud, 1963). Early developmental inability to synthesize adequate thyroid hormone leads to altered physical and mental development (cretinism) (DiGeorge, 1992; Goldey et al., 1995).

In the U.S., most cases of hypothyroidism are associated with some autoimmune problem. Symptoms and signs of hypothyroidism readily prompt patients to seek medical attention. The goal of therapy is to bring persons back into normal thyroid-pituitary balance which, secondarily, greatly minimizes any potential for carcinogenic effects. Overt hypothyroidism, with reduced thyroid hormone and increased TSH levels, requires treatment; it has an incidence of about 0.2% in women, less in men. Subclinical hypothyroidism may have an incidence of 5% among women; men are affected less often; and incidence increases significantly with age. It is not agreed as to whether these people need treatment (Tunbridge & Caldwell, 1991). Because of the nonspecific nature of certain symptoms of hypothyroidism, some persons may go for a length of time before diagnosis and treatment.

Seemingly, the thyroid status of persons living in an iodide-deficient area or those who are hypothyroid due to other causes might be made worse from significant exposure to naturally occurring chemicals or xenobiotics that can further disrupt thyroid-pituitary functioning (Eltom et al., 1985; Hasegawa et al., 1991). The possible consequences of chemical exposure on this subpopulation of individuals may warrant consideration on a case-by-case basis. To the extent that data exist in humans as to their thyroid-pituitary status, one should carefully review central value parameters as well as the distribution of values between chemically exposed and unexposed groups. Analyses may include: (a) whether the chemical or chemicals affect the same or different

sites of antithyroid action; (b) the ways various antithyroidal and other effects might combine to influence potential cancer risks by the same and different routes of exposure, using the guidance in the EPA mixtures assessment guidelines (U.S. EPA, 1986b); (c) whether there may be modes of action other than some antithyroid means; (d) the composition of human populations; (e) the numbers and nature of sensitive individuals; (f) the magnitude and pattern of chemical exposure; and (g) estimates of risk to the general population and to subpopulations with some potential increase in sensitivity.

It is recognized that the human thyroid is susceptible to ionizing radiation, the only verified human thyroid carcinogen. Children are known to be more sensitive than adults to the carcinogenic effects of radiation (NRC, 1990; IAEA, 1996). The major effect of ionizing radiation on the thyroid is thought to be due to mutation. Antithyroid effects can also be induced at elevated radiation doses due to cytotoxicity of follicular cells with resulting reduction in thyroid hormone and elevation of TSH. Mutagenic chemicals, however, do not act totally like radiation:

(a) X rays penetrate the body and target organs without having to be absorbed. Chemicals must be absorbed and distributed to target organs.

(b) Unlike most organic chemicals, radioiodine is actively transported and concentrated in the thyroid gland, and it becomes incorporated into nascent thyroglobulin.

(c) Given that the size of the thyroid gland is smaller in children than in adults, for a given blood level of radioiodine, the internal dose to the thyroid of a child is greater than that for an adult.

(d) Radioiodine in the Chernobyl accident was picked up by cattle and incorporated into milk. Due to differences in milk consumption, the external dose presented to children was greater than to adults. Thyroid cancer inducing chemicals may or may not be found in foods differentially consumed by children.

(e) Single quanta of radiation result in a series of ionizations within biological material, each of which can react with DNA to induce mutations and affect the carcinogenic process. Chemicals are much less efficient: they frequently need to be metabolized to active intermediates, with each molecule interacting singly with DNA, usually by forming adducts which can be converted to mutations.

(f) The spectrum of mutagenic effects vary with the source. Ionizing radiation often results in deletions and other structural chromosomal aberrations, while chemicals not uncommonly produce more gene mutations.

(g) The thyroid of children is more sensitive to carcinogenic effects of external radiation on a per unit dose basis than in adults, especially for children less than 5 years of age.

Sensitivity decreases with advancing age and seems to disappear in adulthood. It is estimated that, overall, children may be two or more times more sensitive to carcinogenic effects of external emitters than are adults (NRC, 1990).

The relative sensitivity of children to mutagenic chemicals that produce thyroid tumors in rodents is totally unknown. Certainly ionizing radiation is a special risk factor for children. Recognizing that direct acting chemicals can produce mutations, it seems possible that they may have enhanced carcinogenic effects in children. To the extent possible, the role of mutagenic agents in general as well as agent specific information on thyroid carcinogenesis should be evaluated from qualitative as well as quantitative standpoints in risk assessments. This information should be conveyed to risk managers so that they can consider it in decision making. It would seem possible that chemicals producing mutagenic effects like radiation may pose some accentuated risk to children. More research is needed in this area.

3. HIGHLIGHTS OF CASE STUDIES

To illustrate the risk assessment guidance for thyroid tumors that is developed in this science policy, four detailed case studies of *hypothetical chemicals* are presented in appendix A. Each case study indicates the types of data that might be available on chemicals and the process that can be employed in assessing their significance. Attention is placed on determination of whether the observed thyroid tumors may be the consequence of thyroid-pituitary imbalance and of ways to project potential dose-response relationships. A summary only of those cases is presented here (table 7).

Table 7. Case study summaries: dose-response assessments

Compound	Antithyroid Effect	Antithyroid Site	Mutagenic	Other tumors	Thyroid dose response MOE[a]	Thyroid dose response Linear	Comment
1. Thionamide	yes	thyroid, periphery	no	no	yes	no	Detailed antithyroid data; no mutagenic effect
2. Chlorinated cyclic hydrocarbon	yes	liver (?)	no (?)	no (?)	no	yes	Multiple data gaps; need more information
3. Bis-benzenamine	yes	thyroid	yes	yes	yes	yes	Antithyroid and mutagenic; multiple tumor sites
4. Nitrosamine	no	not applicable	yes	yes	no	yes	No antithyroid effect

[a]MOE = margin of exposure.

3.1. COMPOUND 1: A THIONAMIDE THAT AFFECTS THE SYNTHESIS OF ACTIVE THYROID HORMONE: THYROID PEROXIDASE AND 5'-MONODEIODINASE INHIBITION

Compound 1 is a thionamide that causes thyroid tumors in both sexes of two rodent species and pituitary tumors in one species; no other tumors are increased in animals, and no tumor effects have been noted in humans. Mutagenic activity is not expected to play a role in its carcinogenicity given its chemical structure and the results of short-term testing.

The thyroid and pituitary tumors produced are thought to be the result of alterations in thyroid-pituitary functioning because the compound inhibits (1) thyroid hormone synthesis in the thyroid and (2) conversion to the active form in the periphery. As a result, circulating thyroid hormone levels decrease and TSH levels increase, which stimulate thyroid cells to proliferate and eventually develop tumors. The process is reversible at least early in its course.

The compound acts as a promoter in a thyroid initiation-promotion study. Increases in thyroid cells do not develop following chemical dosing when thyroid-pituitary balance is maintained by coadministration of exogenous thyroid hormone. The pituitary tumors synthesize TSH, as would be expected given the negative thyroid-pituitary feedback loop.

Dose-response information was available for multiple endpoints spanning from 28 days to chronic administration in rats; consistent findings were noted, which increases the confidence in the data set. In the absence of a mechanistic model, dose-response relationships were evaluated using the most sensitive indicator, TSH levels, from a 28-day rat study to estimate an NOAEL. A simple linear extrapolation was made from observed TSH levels associated with doses of compound 1 down to those within the control group range. This generates a point of departure (NOAEL estimate) for calculation of margins of exposure. No information is available to evaluate directly the carcinogenic effects of compound 1 in humans. In regard to thyroid-pituitary status, exposed workers show no indication of imbalance, and monkeys appear less sensitive on a mg/kg basis than rats.

3.2. COMPOUND 2: A CHLORINATED CYCLIC HYDROCARBON THAT MAY INFLUENCE THYROID THROUGH EFFECTS ON THE LIVER; SIGNIFICANT DATA GAPS

Compound 2 produces a significant dose-related increase in thyroid tumors but not tumors at other sites in rats. The agent has not been tested for carcinogenicity in a second rodent species. Some structural analogues have produced mouse liver tumors but not thyroid tumors.

The agent is nonmutagenic for *Salmonella* gene mutations; it has not been tested in any other short-term tests. Some analogues of the chemical produce structural chromosome aberrations. Short-term administration of compound 2 to rats leads to enlargement of the thyroid gland and reductions in T_3 and T_4; TSH levels have not been measured. The chemical induces liver microsomal enzymes.

Major data gaps preclude a definitive evaluation of the carcinogenic potential of compound 2 and the cause of the thyroid tumors. Further research is encouraged to discern the possible cause of the tumors to see if they may be due to interference of thyroid-pituitary

functioning or mutagenic properties. Because of the data gaps and uncertainties in the case, a low-dose linear extrapolation should be used until a more complete database is developed.

3.3. COMPOUND 3: A BIS-BENZENAMINE THAT PRODUCES THYROID AND LIVER TUMORS; ANTITHYROID AND MUTAGENIC EFFECTS

Compound 3, a bis-benzenamine, produces thyroid follicular cell tumors in rats and mice and liver tumors in mice. The thyroid tumors are associated with thyroid-pituitary derangement; there is thyroid cell and gland enlargement presumably due to inhibition of thyroid peroxidase activity, and there is indication that thyroid hormone levels are reduced and TSH levels are increased. Such effects could account for the thyroid tumors. However, compound 3 also is DNA reactive and produces gene mutations and structural chromosome aberrations in short-term test systems. These DNA effects could condition tumor development in both the thyroid and liver. Dose-response relationships should include consideration of the DNA-modulated effects for the liver tumors. For the thyroid gland, the DNA-associated effects could be used to place an upper bound on the risks, whereas nonlinear considerations might be used to project a lower bound on "cancer risk."

3.4. COMPOUND 4: A NITROSAMINE THAT IS MUTAGENIC AND HAS NO ANTITHYROID EFFECTS

Compound 4, a nitrosamine, produces thyroid follicular cell tumors in rats after a very short latency period. It also produces lung, liver, and kidney tumors in rats after a short latency period and pancreatic, liver, and lung tumors in Syrian hamsters. Compound 4 is mutagenic in various short-term tests. Because compound 4 is mutagenic, causes both thyroid and other tumors with a short latency, and does not cause antithyroid effects, the thyroid follicular cell tumors appear to be caused by a mutagenic mode of action. Dose-response relationships for the thyroid tumors should be evaluated using a low-dose linear default procedure.

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