

US EPA ARCHIVE DOCUMENT

APPENDIX A

CASE STUDIES OF COMPOUNDS

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APPENDIX A: CASE STUDIES OF COMPOUNDS

COMPOUND 1: A THIONAMIDE THAT AFFECTS THE SYNTHESIS OF ACTIVE THYROID HORMONE; THYROID PEROXIDASE AND 5'-MONO-DEIODINASE INHIBITION

EXECUTIVE SUMMARY

Compound 1 may pose a human thyroid carcinogenic hazard at high exposures that might be expected to produce disruptions in the thyroid-pituitary feedback loop. Given the extensive mode-of-action information showing that the thyroid follicular cell tumors are produced by stimulation of the thyroid by thyroid-stimulating hormone (TSH) and not due to mutagenic action, default nonlinear considerations will be employed to estimate concerns for human exposure.

Compound 1 causes thyroid tumors in two rodent species and pituitary tumors in one species, as do other members of this chemical class; no other tumor incidences are increased. There are no tumor findings in humans. Mutagenicity is not expected to play a role in its carcinogenicity; it acts as a promoter of thyroid tumors in an initiation-promotion protocol. The tumors are thought to be the result of alterations in the thyroid-pituitary feedback: the chemical inhibits thyroid hormone synthesis in the thyroid and conversion to the active form in the periphery. Lowered thyroid hormone levels induce the pituitary to increase TSH levels (and to enlarge), which stimulate thyroid cells to enlarge in size, to increase in number, and finally to develop tumors. The process is reversible at least early in its course, and thyroid hyperplasia does not develop following chemical dosing when thyroid-pituitary balance is maintained by exogenous thyroid hormone administration. The pituitary tumors synthesize TSH, as would be expected given the negative thyroid-pituitary feedback loop.

Dose-response relationships are evaluated using the most sensitive indicator from repeat dose, subchronic, and chronic studies, that is, TSH levels from a 28-day rat study. A simple interpolation is made from observed TSH levels associated with doses of compound 1 down to the midpoint of the control group. This generates a point of departure 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 humans show no thyroid imbalance, and monkeys appear less sensitive on a mg/kg basis than rats. Thus, the estimated point of departure from the rat study is probably a conservative estimate for compound 1.

DETAILED DATA

1. Cancer Findings

In chronic rodent toxicity tests, groups of F344 rats and B6C3F₁ mice were administered compound 1 in the feed for 2 years to produce doses of 120, 240, and 480 mg/kg/day in rats and 240, 480, and 960 mg/kg/day in mice. The study design was adequate, and survival of the animals was sufficient at all doses. Results are summarized below and in tables A-1 and A-2.

Table A-1. F344 rats with thyroid follicular cell tumors in a 2-year study of compound 1

Incidence (percent) in males and females according to dose (mg/kg/day)								
Dose	0		120		240		480	
Sex	M	F	M	F	M	F	M	F
Follicular adenoma	1/49 (2)	0/50	1/49 (2)	0/50	10/47* (21)	14/48* (29)	34/45* (76)	28/46* (61)
Follicular carcinoma	1/49 (2)	0/50	0/49	0/50	4/47* (9)	3/48* (6)	8/45* (18)	7/46* (15)
Adenoma + carcinoma	2/49 (2)	0/50	1/49 (2)	0/50	14/47* (30)	17/48* (35)	42/45* (93)	35/46* (76)

* $p < .05$ compared to control value.

Table A-2. B6C3F₁ mice with thyroid follicular cell tumors in a 2-year study of compound 1

Incidence (percent) in males and females according to dose (mg/kg/day)								
Dose	0		240		480		960	
Sex	M	F	M	F	M	F	M	F
Follicular adenoma	1/48 (2)	2/50 (4)	1/46 (2)	1/47 (2)	2/44 (5)	1/47 (2)	16/47* (34)	11/46* (24)
Follicular carcinoma	0/48	0/50	0/46	0/47	0/44	0/47	0/47*	1/46* (2)
Adenoma + carcinoma	1/48 (2)	2/50 (4)	1/46 (2)	1/47 (2)	2/44 (5)	1/47 (2)	16/47* (34)	11/46* (26)

* $p < .05$ compared to control value.

Thyroid tumor frequency in rats and mice was elevated by compound 1. In both male and female rats, the incidences of thyroid follicular cell adenomas, follicular cell carcinomas, and both tumors combined were statistically significantly increased at the middle and high doses (table A-1). Thyroid follicular cell adenomas but not carcinomas were increased significantly in mice at the highest dose (table A-2). Adenomas of the anterior lobe of the pituitary gland were significantly increased in high-dose male rats (19/45 vs. 10/49 in control) and female rats (27/46 vs. 18/50 in control) and in high-dose male mice (8/47 vs. 1/48 in control). Tumor incidence was not elevated at any other organ site in any species-sex combination.

In conclusion, the tumor data provide solid evidence of a tumorigenic effect of compound 1 for the thyroid in both sexes of rat and mouse as well as a weak effect on the pituitary in rats and male mice.

2. Mechanistic Considerations

a. Mutagenicity

This compound did not demonstrate mutagenic effects in the *Salmonella* mutation assay in a set of tester strains both for frameshift and base-pair interactions, with and without metabolic activation. Point mutation tests in *Saccharomyces* were negative. In mammalian cells in culture,

the mouse lymphoma test produced negative results, but there were weakly positive results for the frequency of sister chromatid exchanges in CHO cells. In in vivo tests, compound 1 was negative for chromosomal aberrations in the rat bone marrow and mouse dominant lethal tests. The compound was investigated for DNA reactivity in rat liver cells in vivo using ^{14}C - and ^{35}S -labeled compound. There was no evidence of DNA binding in these cells at the limit of sensitivity. *Saccharomyces* was studied for mitotic gene conversion and gave a weak positive indication of effect. Even though there is some genetic activity in a fungal test system and a mammalian cell test, these test systems are not indicators of mutagenicity and are difficult to interpret as to their significance for a cancer mode of action. In addition, given the lack of DNA reactivity and gene and structural chromosomal mutations, it is concluded that there is an overall lack of mutagenic activity for compound 1 relevant to the evaluation of cancer.

b. Thyroid Growth

Following the results in the chronic toxicity studies, the company designed an extensive 28-day study in male rats that included the collection of information on thyroid weight and hormone levels at seven different doses (15 rats/dose). The mean thyroid weights were significantly increased at the three highest doses (table A-3).

Table A-3. Mean thyroid weights (mg) in male F344 rats after treatment with compound 1 for 28 days

Dose (mg/kg/day)							
0	15	30	60	120	240	480	960
21.4 ±0.6	22.0 ±0.7	22.1 ±0.8	22.7 ±0.7	23.9 ±0.9	29.9* ±1.9	48.0* ±2.6	48.6* ±3.9

* $p < .05$ compared to control value.

Thyroid weight data were also available from male and female rats in the 2-year study (including its 12-month interim sacrifice). The observations from these studies were consistent with the information derived from the 28-day study in male rats. As an example, thyroid weights were significantly increased at the 240 and 480 mg/kg/day dose levels in male and female rats sacrificed at the interim period of 12 months in the cancer bioassay (table A-4). In all of these studies on thyroid weight, expressing the thyroid weight data as a percentage of body weight (relative thyroid weight) produced exactly the same results, indicating that the thyroid weight gain was chemically induced and not just a normal growth change.

The increase in thyroid weight at the different times correlated with a progressive increase in hypertrophy and hyperplasia of the thyroid follicular cells, noted histologically. These changes were diffuse throughout the gland, as opposed to focal. Hypertrophy was characterized by an increase in size of cells lining the follicles from the normal flattened or low cuboidal shape to columnar. Hyperplasia involved a generalized increase in the number of follicular cells and the number of follicles. Increased cellularity was accompanied by a reduction in the size of the follicles and in colloid content. Hyperplastic follicles were often irregular in shape with a narrowed, slitlike lumen. The follicular epithelium was often convoluted, sometimes projecting as papillary formations into the follicular lumen. These changes were more pronounced centrally within the gland than at the periphery. Nodular hyperplasia was reported at one or more sites in animals in the top two doses. The incidence of hyperplasia at 12 months in male and female F344 rats is summarized in table A-5.

In the 28-day study, the mitotic index for follicular cells was determined in the male rats receiving doses of 480 mg/kg/day by manually counting mitotic figures (metaphases) in standard

Table A-4. Mean thyroid weights (mg) in F344 rats treated with compound 1 and sacrificed at 12 months

Dose (mg/kg/day)				
	0	120	240	480
Males	29.3±2.1	29.4±2.7	44.2±3.8*	52.6±3.2*
Females	25.3±3.2	25.1±2.1	34.3±2.3*	44.7±2.0*

**p*<.05 compared to control value.

Table A-5. Incidence of thyroid follicular cell hyperplasia in rats at 12 months

Dose (mg/kg/day)				
	0	120	240	480
Males	0/15	0/15	10/15*	12/15*
Females	0/15	0/15	8/15*	11/15*

**p*<.05 compared to control value.

unit areas of thyroid tissue. The mitotic index, expressed as the number of cells in metaphase per 10,000 nuclei, was increased fivefold over the untreated control rats (control value 1.5±0.3 vs. treated group value 7.8±0.2), providing further evidence of thyroid follicular cell proliferative

activity in response to compound 1. Data on thyroid weights and morphology from a 13-week oral study with rhesus monkeys were provided (table A-6). Males (four per group) were administered a dose of compound 1 (300 mg/kg/day) that was above those producing thyroid tumors and weight changes in rats on a mg/kg basis. There were no treatment-related effects on thyroid weights, nor were there any differences in gross and histologic observations of the thyroid between treated and control monkeys. Specifically, follicular cell hyperplasia, either diffuse or focal, was not observed at either dose of compound 1. Additionally, all clinical chemistry determinations in the monkeys were within the normal range.

In conclusion, the data on thyroid weights and morphology show that compound 1 has a specific effect on the thyroid in rats, increasing thyroid size through stimulation of cellular hypertrophy and diffuse follicular cell hyperplasia. Limited data from monkeys suggest that primates may be less sensitive to these thyroid effects than rats. The sensitivity of mice requires more study.

Table A-6. Mean thyroid weights (gm) in rhesus monkeys after treatment with compound 1 for 13 weeks

Dose (mg/kg/day)		
	0	300
Males	0.57 +0.3	0.45 ±0.2

c. Hormones

As part of the mechanistic studies on compound 1, T₃ (triiodothyronine), T₄ (thyroxine), and TSH levels were measured in male rats from the 28-day study at all seven dose levels. Table A-7 presents mean levels (15 animals per group). Serum T₄ and T₃ levels were significantly lower than controls in the three higher doses tested but not the four lower doses. TSH levels were significantly elevated in a dose-related manner at doses of 120 mg/kg/day and above; no such increases were noted in the two lowest doses, which were comparable to the control. Comparable changes in serum hormone levels were noted at the same doses in the interim sacrifice of the 2-year rat study. These hormone changes along with the alterations in thyroid histology noted above provide unequivocal evidence that compound 1 influences thyroid-pituitary functioning.

d. Site of Action

(1) **Thyroid.** To provide data on the site of perturbation of the thyroid-pituitary axis, information was obtained on the effect of compound 1 on thyroid peroxidase. Hog thyroid peroxidase activity was inhibited in vitro at several dose levels through a reversible interaction not involving covalent binding and suicide inactivation, as has been reported for some of the other thionamides.

(2) **Other.** Another study showed that compound 1 is also capable of inhibiting 5'-monodeiodinase activity. The effect of compound 1 on the enzymatic conversion of T₄ to the active form, T₃, was investigated by incubating the supernatant fractions (containing 5'-deiodinase) from perfused livers of control rats and rats treated with three i.p. doses of compound 1. The amount of T₃ generated in the incubation mixture was a measure of enzyme activity; inhibition of the enzyme results in decreased generation of T₃ and increased production of the inactive form, reverse T₃ (rT₃). The results (table A-8) indicate a dose-dependent inhibition of

Table A-7. Serum T₃, T₄, and TSH levels in male F344 rats after treatment with compound 1 for 28 days

	Dose (mg/kg/day)							
	0	15	30	60	120	240	480	960
T ₃ (ng/dL)	56.2 ±3.3	58.1 ±2.4	61.2 ±3.1	63.5 ±2.4	59.7 ±3.3	44.4* ±2.5	26.4* ±2.6	23.5* ±2.0
T ₄ (µg/dL)	3.4 ±0.2	3.3 ±0.1	3.6 ±0.2	3.4 ±0.2	3.1 ±0.1	2.5* ±0.3	0.6* ±0.1	0.2* ±0.1
TSH (ng/ml)	3.3 ±0.4	3.0 ±0.5	3.6 ±0.4	3.9 ±0.6	5.9* ±0.7	7.8* ±0.9	12.2* ±0.7	13.8* ±1.0

**p*<.05 compared to control value.

Table A-8. Effect of compound 1 on hepatic 5'-monodeiodinase in rats

Dose (mg/kg/day)	Hepatic T₃ generation from T₄ (% of control value)
0	100
125	97.5
250	61.0*
500	39.5*

* $p < .05$ compared to control value.

5'-deiodinase activity by compound 1 and provide evidence of a mechanism for reducing effective thyroid hormone levels and enhancing TSH production. Collectively, these mechanistic data confirm the position that compound 1 exerts actions centrally on the thyroid and peripherally (e.g., liver) to reduce thyroid hormone formation.

e. Dose Correlations

The plethora of data on compound 1 lead to some important correlations between dose and various neoplastic and preneoplastic lesions as well as other effects. They are discussed later in the Hazard and Dose-Response Characterization section of this appendix; specific correlations are illustrated in figures A-1 and A-2.

Figure A-1
Thyroid Weight and Tumor Responses
to Compound 1

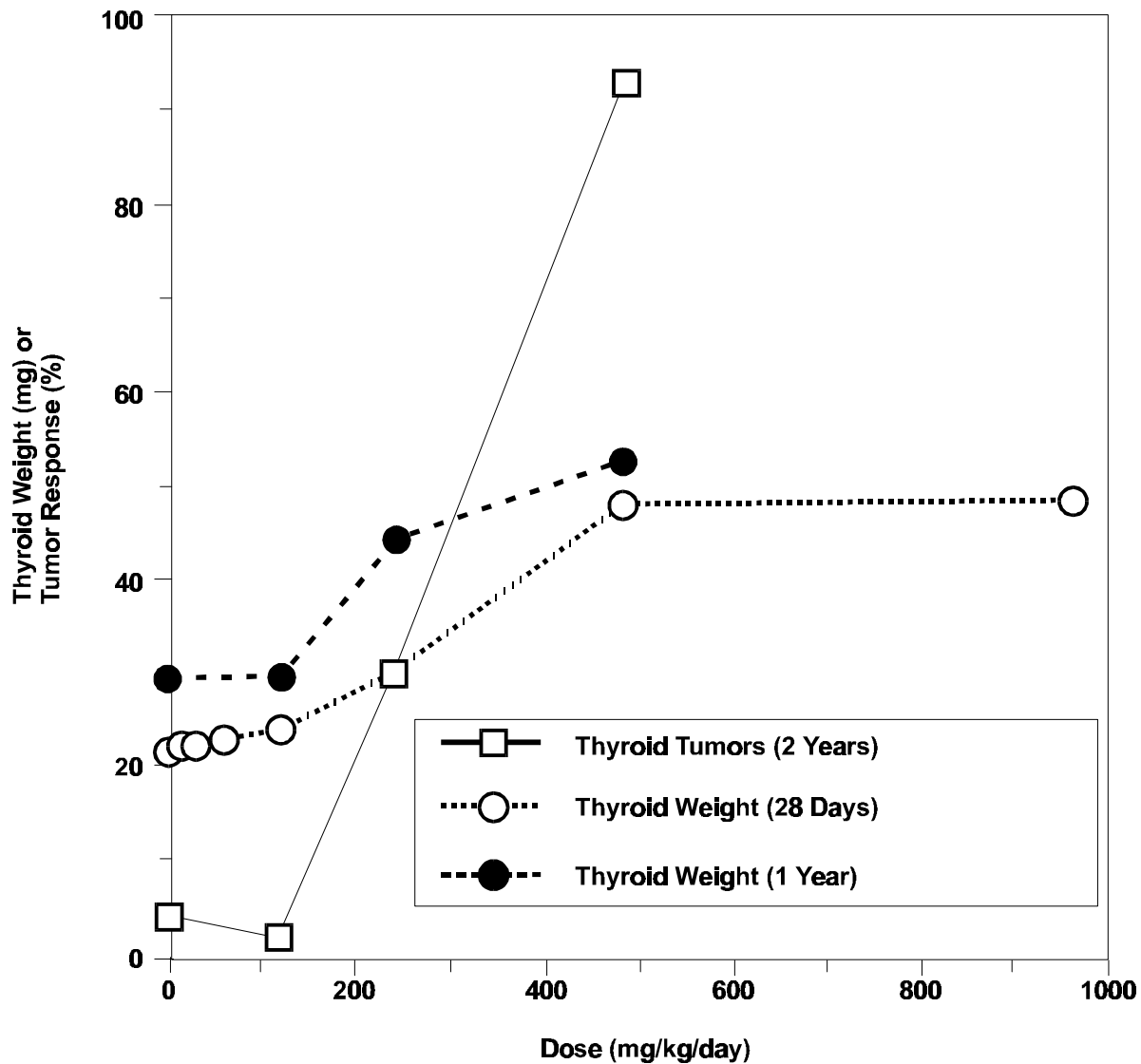
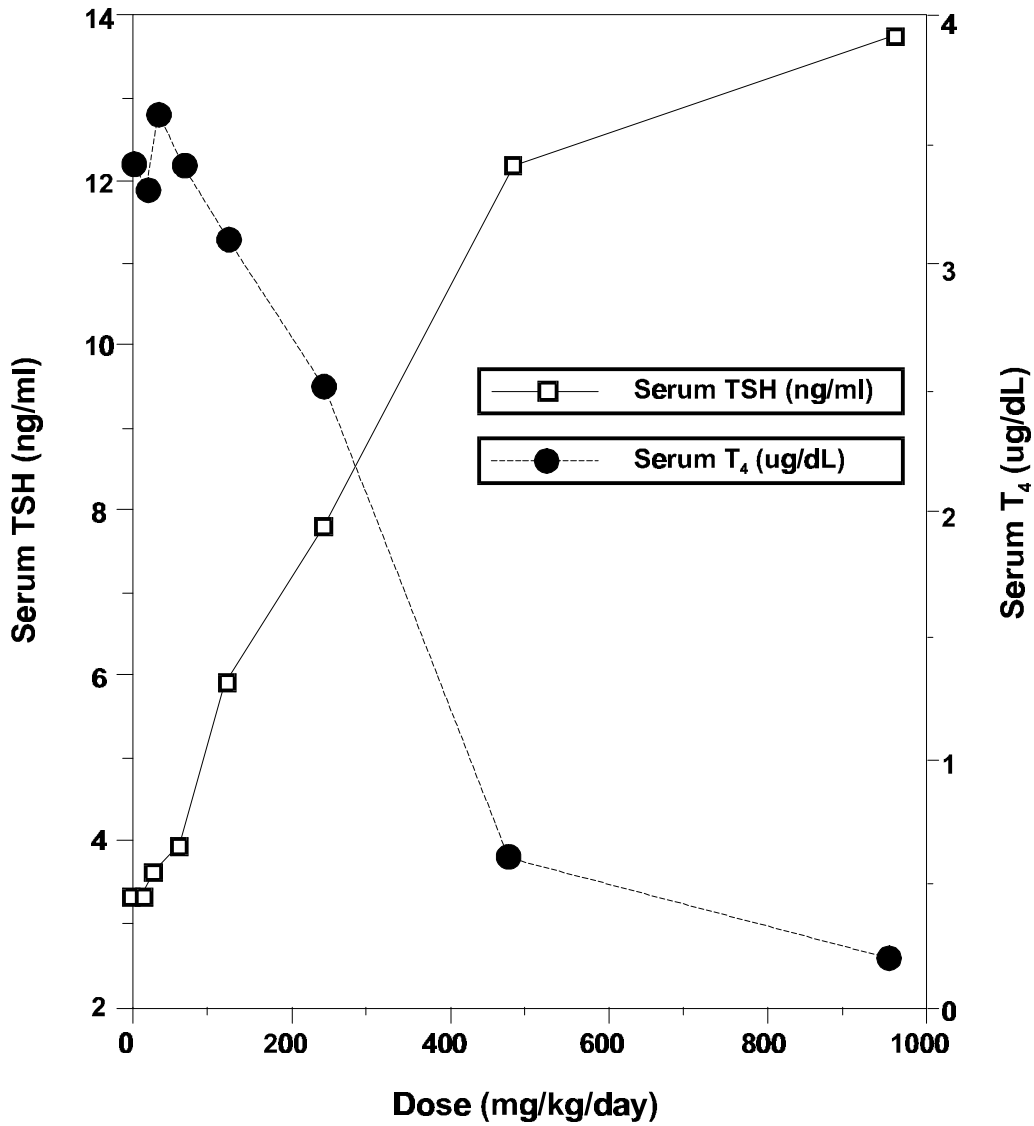


Figure A-2
Hormone Responses to Compound 1



f. Ancillary Data

Data were provided on reversibility of induced effects, pituitary changes and initiation-promotion aspects for compound 1 in support of the position that the thyroid tumors occurred as the result of thyroid-pituitary disruption.

(1) **Reversibility studies** . Male rats were administered 240 mg/kg/day of compound 1 for 4 weeks to induce increases in thyroid weight and hormone changes. Animals were then allowed to recover for 4 additional weeks, by which time thyroid weights and serum T_3 and T_4 and TSH had returned to the control range. This study demonstrates that cells are not irreversibly committed to progress to neoplasia. Instead, weight and thyroid hormone changes are completely reversible to preexposure values upon cessation of treatment.

(2) **Effect of exogenous thyroid hormone** . In conjunction with the study described in d(1) above, additional groups of male rats were administered compound 1 (240 mg/kg/day) for 4 weeks in combination with equal amounts of T_4 and T_3 at three different dose levels. Thyroid weights and serum T_4 , T_3 , and TSH levels were measured at the end of the 4-week period. The results, shown in table A-9, are consistent with an interpretation that T_4/T_3 treatment blocks the antithyroid effects of compound 1.

These two studies emphasize the effect of compound 1 on circulating TSH levels as an ultimate mechanism that can be manipulated by a recovery phase or replacement therapy back to the normal range of thyroid-pituitary functioning.

(3) **Initiation-promotion data** . The carcinogen N-bis(2-hydroxypropyl) nitrosamine (DHPN) is used frequently as a model initiator in rodent studies involving the thyroid as a target organ. In one of these studies, male F344 rats, 6 weeks old, were administered a single i.p. injection of DHPN and fed compound 1 in the diet (240 mg/kg/day), beginning 1 week after injection, for 19 weeks. The incidence of thyroid follicular cell tumors in the group receiving DHPN and compound 1 was 95% compared with 5% in the DHPN only group and 0% in the compound 1 only group. The results, summarized in table A-10, provide convincing evidence that compound 1 can act as a promoting agent for the development of thyroid tumors in rats. Alone, the compound did not induce tumors at the end of the observation period, but following a mutagenic initiator a significant proportion of animals developed tumors.

Table A-9. Effect of T₃ and T₄ treatment on thyroid weights and serum T₄, T₃, and TSH levels in male rats given compound 1 over a 28-day period

Doses					
Compound 1 (mg/kg/day)	0	240	240	240	240
T ₃ + T ₄ (μg/kg/day each)	0	0	10	30	50
Thyroid weight (mg)	18.4 ±0.9	27.3* ±0.9	22.2 ±1.7	27.6 ±0.9	17.5 ±0.8
T ₃ (ng/dL)	60.2 ±2.8	45.5* ±2.8	50.7* ±2.1	66.3 ±3.1	63.1 ±1.7
T ₄ (μg/dL)	3.9 ±0.1	2.5* ±0.4	2.9 ±0.5	4.3 ±0.1	4.5 ±0.3
TSH (ng/ml)	3.01 ±0.6	6.36* ±0.7	4.84* ±0.7	3.08 ±0.6	2.62 ±0.4

**p*<.05 compared to control value.

Table A-10. Numbers (percent) of male rats with thyroid tumors at 20 weeks after treatment with DHPN followed by compound 1

Treatments				
	None	DHPN	Compound 1	DHPN + Compound 1
Thyroid tumors	0/20 (0)	1/20 (5)	0/20 (0)	19/20 (95)

(4) Pituitary changes. Because of the finding of pituitary adenomas in the 2-year rat and mouse studies, male rats in the expanded 28-day study were investigated as to the histologic origin of the pituitary lesions. At weekly intervals, groups of animals were sacrificed, and sections of pituitary gland were stained with the aldehyde-thionine-PAS stain or immunohistochemically with an antirat TSH antibody using the peroxidase staining method. Microscopic evaluation of aldehyde-thionine-PAS stained sections showed hypertrophy and hyperplasia of basophilic cells (thyrotrophs) that secrete TSH after 1 week. Immunohistochemical staining of pituitary sections showed a quantifiable increase in TSH immunoreactivity by 7 days. An increase in the number of

thyrotrophs induced by compound 1 correlates with an expected stimulation of the pituitary under conditions of reduced thyroid hormone levels and with increased production of TSH; it is a further indication of an effect on the thyroid-pituitary axis. Accordingly, it is reasonable to conclude that the pituitary adenomas arose from basophilic thyrotrophs.

g. Structure-Activity Relationships

Chemically, compound 1 is a thionamide, a class of compounds that has produced thyroid tumors in rodents and sometimes liver tumors in mice. The chemicals frequently are inhibitors of thyroid peroxidase, and some are also inhibitors of hepatic 5'-monodeiodinase activity. The biological and tumor findings with compound 1 are consistent with these structural attributes.

h. Metabolic and Pharmacokinetic Properties

Metabolism and excretion studies in the rat were conducted with ^{14}C -labeled compound 1 administered orally or intravenously. No comparative studies have been done in the mouse or the monkey. The results, reported in the literature, were in agreement regardless of route of administration. Radioactivity in rat blood was identified as unchanged chemical. Less than 5% of radioactivity was detected in the feces, but 80% to 85% of the administered dose was excreted via the kidneys within 24 hours, all radioactivity clearing by 48 hours. About 15% of the administered dose was excreted in the urine as the unchanged compound, while the major urinary metabolite was the chemical conjugated with glucuronide. Approximately 15% of the compound was excreted into the bile as a glucuronide conjugate (different from the major urinary metabolite), indicating enterohepatic circulation. No oxidative metabolites could be demonstrated in urine or plasma.

Accumulation of compound 1 in the thyroid was investigated by administering the chemical radiolabeled with ^{35}S i.p. to rats and comparing the radioactivity in the thyroid to the serum. The results of this study showed that the intact parent compound preferentially accumulated in the thyroid gland, as has been found for some of the other thiol-containing chemicals and which is consistent with some direct antithyroidal action for compound 1.

3. Human Observations

The manufacturer of compound 1 has produced the chemical, using batch processing, for many years. The medical department monitored thyroid function of about 150 employees directly involved in the synthetic process every 2 years over the preceding 10 years. Overall, the results showed no significant differences between exposed workers and normal values reported in the

literature for serum T_4 and TSH. T_3 , measured in the most recent surveillance, was also within normal limits. No worker exposed to compound 1 reported symptoms or showed signs consistent with hypothyroidism, and medical examination did not reveal any enlarged thyroid glands. Occupational exposure levels never reached the level of quantification using a rather insensitive air monitoring system.

HAZARD AND DOSE-RESPONSE CHARACTERIZATION

Compound 1 may pose a carcinogenic hazard to the human thyroid if doses perturb thyroid-pituitary homeostasis. It produces thyroid tumors (males/females) and related pituitary tumors (males) in rats and mice. It is readily absorbed following oral exposure, and because of its small size and physical properties, it also should be readily absorbed through the lung and to a lesser extent the skin. Extensive mode-of-action information demonstrates that compound 1 inhibits both the synthesis of thyroid hormone in the thyroid gland and the conversion of thyroid hormone (T_4) to its more active form (T_3) in organs outside the thyroid gland. Given this information and the absence of relevant mutagenicity, it would appear that thyroid risk would be minimal under conditions of thyroid-pituitary homeostasis. Therefore, in the absence of a mathematical model that incorporates mode-of-action data on a chemical like this, a dose-response relationship that involves nonlinear default extrapolation will be projected.

There is strong evidence that compound 1 has carcinogenic activity in laboratory animals. The agent is a thionamide, a group of chemicals that often produces thyroid (and sometimes other) tumors in rodents. Both male and female rats and mice receiving compound 1 in feed showed significant increases in thyroid follicular cell tumor (benign and malignant tumors in rats, benign only in mice) incidence. Rats and male mice also showed an increase in benign pituitary tumors. No other tumors were increased in dosed animals. There is no information on the carcinogenicity of compound 1 in humans.

Overall, mutagenicity studies fail to demonstrate relevant effects. There is no indication from those endpoints that there is some direct mechanistic relationship to carcinogenic processes: gene mutations (*Salmonella* and cultured mammalian cells), structural chromosome aberrations (rat bone marrow), DNA reactivity using radiolabeled compound 1, and analysis of structural analogues. Although other studies (yeast mitotic gene conversion and crossing over and mammalian cell sister chromatid exchange) show at least some indication of a positive response, the implication of chemically induced increases in these effects to carcinogenicity is not well understood. There are no studies of the potential genetic effects of compound 1 in the thyroid per se; such information may be useful.

An extensive range of testing demonstrated that the thyroid tumors may be due to disruption of thyroid-pituitary functioning. The chemical causes a dose-related enlargement of the thyroid gland (goitrogenic effect) and a progression of lesions over time. After 28 days of dosing, individual thyroid cells show enlargement in size (hypertrophy), and the proportion of cells in cell division (mitotic index) increases. In addition, there is an increase in the number of thyroid cells (hyperplasia). These factors combined lead to increased thyroid weights of dosed rats. These effects persist in rats sacrificed at 1 year, and after 2 years of treatment, thyroid tumors are noted.

Studies also indicate that compound 1 results in altered thyroid-pituitary *hormone levels*. By 28 days of treatment, there are profound dose-related reductions in serum T₄ and T₃ and increases in TSH. There is a dose correlation between those that influence both hormone levels and thyroid histology. Hormone and histologic effects are reversible when treated animals are returned to a diet without compound 1. All histologic changes in the above studies occur at doses of 240 mg/kg and higher; all changes in hormones occur at the same doses, except TSH at 28 days, which is significantly increased at 120 mg/kg and higher.

The site of action of compound 1's influence on thyroid-pituitary status seems to be centered on the thyroid gland and extrathyroidal sites. The compound is concentrated in the thyroid gland like some other thionamides and produces reversible reduction in thyroid peroxidase activity (in vitro), the enzyme that results in iodination of tyrosyl moieties and their coupling into thyroid hormones. In addition, there is reduction in liver 5'-monodeiodinase activity (≥ 250 mg/kg), which reduces the normal conversion of circulating T₄ to the cellular active form, T₃. Studies of other potential sites of antithyroid action of compound 1 (e.g., interference of thyroid uptake of inorganic iodide and induction of liver enzymes) have not been conducted. However, the identified influence of compound 1 on thyroid hormone synthesis and conversion can adequately explain the increases in TSH levels and the resulting growth of the thyroid gland.

Other studies indicate that thyroid-pituitary imbalance leads to tumor formation. Rat pituitary adenoma cells contain TSH, which is consistent with reductions in thyroid hormone levels with corresponding increased stimulation of the pituitary to produce TSH. Compound 1 promotes thyroid tumors after treatment with an initiator. In addition, thyroid gland and hormonal changes are blocked by coadministration of compound 1 and amounts of thyroid hormones that maintain normal thyroid-pituitary status.

All mechanistic work is consistent with this chemical producing thyroid and pituitary tumors in rodents by a nonmutagenic mode of action that involves (1) inhibition of the synthesis of active thyroid hormone, (2) feedback stimulation of the pituitary to enlarge and synthesize TSH, and (3) TSH stimulation of growth of the thyroid gland. Although the precise events

accounting for transformation of hyperplastic cells into neoplastic cells are unknown, they seem to occur under conditions of continued TSH stimulation. If exposures to compound 1 can be kept at levels that do not lead to thyroid-pituitary disruption, there would be no stimulus for tumor development in either the thyroid or the pituitary.

Relevant dose-response data demonstrate a consistent alteration in test parameters in rats at doses of at least 120 mg/kg. Few data are available in the mouse other than the finding of tumors at a dose (900 mg/kg/day) higher than in rats; therefore, information in rats is used. Figure A-1 shows dose-response plots of thyroid weight after 28 days, thyroid weight after 1 year, and thyroid tumors after 2 years of dosing. Figure A-2 shows similar dose-response plots for T_4 and TSH levels after 28 days of dosing. Other endpoints could have been depicted, but they add nothing significant to what is depicted here; besides, they show similar dose-response relationships. Tumor incidences are increased at 240 mg/kg, a value consistent with increases in thyroid weight (after both 28 days and 1 year of dosing) and decreases in thyroid hormone levels at 28 days. TSH, however, is significantly elevated at doses of 120 mg/kg and above.

Since TSH plays a pivotal role in thyroid stimulation and there is a progressing doubling of doses to help illustrate the effects of dose and effect, the TSH level is used to estimate a dose of compound 1 that would not be expected to increase the hormone level in rats above that of normal, untreated animals. The TSH response appears to be essentially flat from the control through the 15, 30, and 60 mg/kg/day groups, with a profound upward change in slope from 60 mg/kg/day up to the highest dose tested, 960 mg/kg/day.

The question is how far to extrapolate downward from high doses of compound 1 associated with pronounced increases in TSH to reach a value that probably is without significant effect on the thyroid. Given the prominence of TSH in priming the carcinogenic process in the rodent thyroid, one would not want chemically induced additions to the normal homeostatic levels of TSH. In addition, the information at hand is quite revealing; it seems that some doses of compound 1 are without effect on circulating TSH levels. For instance, doses up to about 60 mg/kg/day do not seem to add to the underlying TSH level in any significant way. It appears that some level of compound 1 is required before it can interfere with thyroid homeostasis, that is, to inhibit both thyroid peroxidase and T_4 deiodination to T_3 , to decrease circulating thyroid hormone levels, and to increase TSH. In recognition of these findings, the 60 mg/kg/day no-observed-adverse-effect level (NOAEL) from the 28-day rat study is a reasonable estimate of a point of departure for projecting concern for human exposures and calculating a margin of exposure.

There is limited information on compound 1 concerning the ability to extrapolate the finding of thyroid tumors in rodents to humans. Workers exposed to undisclosed levels of compound 1

have no indication of perturbation of thyroid-pituitary status; all exposed individuals are without significant physical findings and have relevant hormone values with the same mean and distribution as in the general population. A limited number of monkeys exposed to the compound at 300 mg/kg failed to show any thyroid effects; these findings suggest that primates (and possibly humans) may be less sensitive than rats (possibly, at least fivefold on a mg/kg basis) to the antithyroid effects of the chemical. Therefore, using the midpoint of the control TSH level as a point of departure may be a conservative estimate of the potency of compound 1. This information should be considered in the evaluation of the significance of the calculated margin of exposure for humans.

BIBLIOGRAPHY

The following articles were consulted in developing the data used in the case study of this hypothetical chemical.

Arnold, DE; Krewski, DR; Junkins, DB; et al. (1983) Reversibility of ethylenethiourea-induced thyroid lesions. *Toxicol Appl Pharmacol* 67:264-273.

Botts, S; Jokinen, MP; Isaacs, KR; et al. (1990) Proliferative lesions of the thyroid and parathyroid glands, E-3. In: *Guides for toxicologic pathology. STP/ARP/AFIP*. Washington, DC: Armed Forces Institute of Pathology, pp. 1-12.

Engler, D; Burger, AG. (1984) The deiodination of the iodothyronines and of their derivatives in man. *Endocrine Rev* 5:151-185.

Fullerton, FR; Kushmaul, RJ; Suber, RL; et al. (1987) Influence of oral administration of sulfamethazine on thyroid hormone levels in Fischer 344 rats. *J Toxicol Environ Health* 22:175-185.

Hayden, DW; Wade, GG; Handler, AH. (1978) Goitrogenic effect of 4,4'-oxydianiline in rats and mice. *Vet Pathol* 15:649-662.

Hiasa, Y; Kitahori, Y; Kato, Y; et al. (1987) Potassium perchlorate, potassium iodide, and propylthiouracil: promoting effect on the development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)-nitrosamine. *Jpn J Cancer Res* 78:1335-1340.

Horvath, E; Lloyd, RV; Kovacs, K. (1990) Propylthiouracil-induced hypothyroidism results in reversible transdifferentiation of somatotrops into thyroidectomy cells. A morphologic study of the rat pituitary including immunoelectron microscopy. *Lab Invest* 63:511-520.

Littlefield, NA; Gaylor, DW. (1989) Chronic toxicity/carcinogenicity studies of sulfamethazine

in B6C3F₁ mice. *Fd Chem Toxicol* 27:455-463.

Marchant, B; Alexander, WD; Lazarus, JH; et al. (1972) The accumulation of ³⁵S-antithyroid drugs by the thyroid gland. *J Clin Endocrinol* 34:847-851.

McClain, RM. (1991) Dose response characteristics for non-cancer endpoints responsible for tumor induction in rodents. Presented at ILSI Workshop on Dose-Response Models, Reston, VA, December 5-6, 1991. Washington, DC: International Life Sciences Institute.

Mohr, U; Reznik, G; Pour, P. (1977) Carcinogenic effects of diisopropanolnitrosamine in Sprague-Dawley rats. *J Natl Cancer Inst* 58:361-366.

Nogimori, T; Braverman, LE; Taurog, A; et al. (1986) A new class of propylthiouracil analogues: comparison of 5'-deiodinase inhibition and antithyroid activity. *Endocrinology* 118:1598-1605.

Owen, NV; Worth, HM; Kiplinger, GF. (1973) The effects of long-term ingestion of methimazole on the thyroids of rats. *Fd Cosmet Toxicol* 11:649-653.

Sitar, DS; Thornhill, DP. (1972) Propylthiouracil: absorption, metabolism, and excretion in the albino rat. *J Pharmacol Exp Therap* 183:440-448.

Steinhoff, D; Weber, H; Mohr, U; et al. (1983) Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters. *Toxicol Appl Pharmacol* 69:161-169.

Swarm, RL; Roberts, GKS; Levy, AC; et al. (1973) Observations on the thyroid gland in rats following the administration of sulfamethoxazole and trimethoprim. *Toxicol Appl Pharmacol* 24:351-363.

Thomas GA; Williams, ED. (1991) Evidence for and possible mechanisms of non-genotoxic carcinogenesis in the rodent thyroid. *Mutat Res* 248:357-370.

Wynford-Thomas, D; Stringer, BMJ; Williams, ED. (1982) Goitrogen-induced thyroid growth in the rat: quantitative morphometric study. *J Endocrinol* 94:131-140.

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

EXECUTIVE SUMMARY

Compound 2 is a chlorinated cyclic hydrocarbon that produces thyroid but no other tumors following chronic dosing in male and female rats. Some structural analogues produce liver tumors in mice; none has produced thyroid tumors.

In studies on the mode of carcinogenic action, compound 2 produces enlargement of the thyroid gland and a decrease in serum T₄ but not T₃. There is an increase in liver weight in rats in the chronic study. Induction of liver microsomal enzymes is reported in one acute study using a very high dose. Compound 2 is nonmutagenic in the only test in which it has been evaluated (*Salmonella* gene mutation); however, some analogues produce structural chromosome aberrations.

Many uncertainties are associated with the assessment of compound 2. Major data gaps include a cancer study in a second species, further mutagenicity testing, demonstration of the chemical's influence on TSH levels, and a delineation as to whether enhanced thyroid metabolism and excretion by the liver may account for the observed thyroid tumors.

Given the data at hand, a low-dose linear approach is used for dose-response purposes until such time that further data are developed and a mode of action is determined.

DETAILED DATA

1. Cancer Findings

The animal cancer bioassay provides convincing evidence of a positive tumorigenic effect by compound 2 for the thyroid in rats. This study was conducted in male and female rats, 50 animals per group, using three dose levels of compound 2 (15, 50, and 150 mg/kg) and a control, administered by gavage each day, seven times weekly. Over the 2-year study period, survival of the animals was excellent, with the high-dose males exhibiting the highest mortality at 10%.

Thyroid tumor incidences with respect to dose are summarized in table A-11. A dose-

related increase in thyroid tumors was statistically significant in the mid- and high-dose males and females.¹ A significant increase in tumors was not detected for any other organ. In

Table A-11. Numbers (percent) of rats with thyroid tumors (adenomas/carcinomas combined)

Sex	Dose (mg/kg/day by gavage)			
	0	15	50	150
Male	2/50 (4)	3/50 (6)	7/48* (15)	7/45* (16)
Female	1/50 (2)	1/50 (2)	7/49* (14)	9/47* (19)

* $p < .05$ compared to control value.

particular, out of a total of 389 rats in this carcinogenicity bioassay, only two liver tumors, both hepatocellular adenomas, were observed, one in a mid-dose male and one in a control female.

2. Mechanistic Considerations

a. Mutagenicity

Compound 2 was nonmutagenic in *Salmonella* assays for frameshift (TA-98) and base-pair (TA-100) mutations. No other endpoints have been assayed.

b. Thyroid Growth

Information on thyroid weight and histology is available from a 90-day study in which compound 2 was administered daily by gavage to male rats, 10 per group. Thyroid weights were increased in a dose-related manner at all doses tested (table A-12). Effects were significant at each dose as compared with the control. Microscopic examination of the animals showed that the increase in thyroid weight was due to diffuse follicular cell hyperplasia, characterized by more numerous but smaller follicles. There was no statement as to whether cells were hypertrophied.

¹ The investigators did not report separate incidences for thyroid adenomas and carcinomas; see table A-11.

c. Hormones

In a 14-day study using male rats (10 per dosage group), the chemical was administered daily by gavage, and serum T₃ and T₄ concentrations were then measured (table A-13). T₄ concentrations showed dose-related decreases and were statistically significant at the two highest doses. T₃ levels were not affected by dosing. There was no measurement of TSH levels.

Table A-12. Mean thyroid weights (mg) in male Sprague-Dawley rats treated for 90 days with compound 2

Dose (mg/kg/day by gavage)			
0	15	50	150
16.4±2.5	23.8±4.1*	24.1±5.8*	32.6±4.6*

**p*<.05 compared to control value.

Table A-13. T₃ and T₄ levels in male Sprague-Dawley rats treated with compound 2 for 14 days

Hormone	Dose (mg/kg/day by gavage)				
	0	5	10	50	150
T ₃ (ng/dl)	94.4±19.1	89.3±15.5	94.5±11.2	90.7±14.8	91.3±10.6
T ₄ (µg/dl)	4.6±1.2	4.5±0.9	4.0±0.6	3.5±0.7*	3.3±0.6*

**p*<.05 compared to control value.

d. Site of Action

(1) **Thyroid.** No studies investigated the effects of compound 2 on the thyroid gland *per se*.

(2) **Other.** There is a lack of information concerning the effects of compound 2 on 5'-monodeiodinase activity.

(3) **Liver.** In the 2-year rat study, the livers in low-, mid-, and high-dose animals were heavier on an absolute and relative-to-body-weight basis than those in controls; increases were statistically significant in the mid- and high-dose groups. There was no mention of histologic changes in the livers.

Compound 2's mixed-function oxidase (MFO)-inducing properties have been

demonstrated in one acute study in Sprague-Dawley rats. Animals received a single dose of compound 2 that was equivalent to one-half the acute LD₅₀ (1,000 mg/kg). Liver microsomal enzyme activities were measured 3 days later and included cytochrome P-450 content, benzphetamine-N-demethylase, 7-ethoxycoumarin O-deethylase, and NADPH-cytochrome c-reductase. In all cases, the values were significantly greater in treated animals than in controls (table A-14).

Table A-14. Hepatic microsomal enzyme activities in male Sprague-Dawley rats treated for 14 days with compound 2

Dose (mg/kg)	NADPH-cytochrome c-reductase (nmol reduced/mg)	Cytochrome P-450 (nmol/mg protein/min)	Benzphetamine-N-demethylase (nmol/mg protein/min)	7-Ethoxycoumarin O-deethylase (nmol/mg protein/min)
0	1.48±0.10	6.54±0.93	2.14±0.30	253.3±70.7
1,000	2.09±0.21*	9.27±0.84*	3.84±0.20*	370.8±51.4*

**p*<.05 compared to control value.

These data provide evidence that compound 2 induces liver enzyme activity in the rat at a high acute dose. No studies have been conducted following repeat dosing and at chemical doses around those that are associated with the development of thyroid tumors. Similarly, there is an absence of data on the activity of uridine diphosphate glucuronosyl-transferase in the liver and of T₄ clearance from serum or excretion in the bile.

e. Dose Correlations

Given the data gaps, only preliminary correlations can be developed between dose and specific endpoints. These are discussed later (see table A-15).

f. Metabolism

In the rat, compound 2 is freely absorbed through the gastrointestinal tract and extensively metabolized in the liver; individual metabolites have not been characterized. Little else is known about the handling of the chemical, and no comparative studies in different species are available.

g. Structure-Activity Relationships

Some structural analogues produce liver tumors in mice, but most have not been studied at all for carcinogenicity. None has induced thyroid tumors. Some analogues produce structural chromosome aberrations in cultured mammalian cells; one close analogue also induces these

aberrations in mammals in vivo.

3. Human Observations

No relevant data are available on thyroid-related function or carcinogenicity of this chemical in humans.

Table A-15. Summary of effects relevant to evaluating the significance of thyroid tumors in rats

Study	Effect	Dose (mg/kg/day)					
		5	10	15	50	150	1,000
2-Year study:	tumors			-	+	+	
	liver weight			±	+	+	
90-Day study:	thyroid weight			+	+	+	
14-Day study:	T ₄ decrease	-	±		+	+	
Acute study:	MFO induction						+

HAZARD AND DOSE-RESPONSE CHARACTERIZATION

Compound 2, a chlorinated cyclic hydrocarbon, produces a small but significant increase in thyroid tumors in gavaged male and female rats. No other tumor increases are noted. The chemical has not been tested for carcinogenicity in a second laboratory species, and nothing is known about its potential carcinogenic effects in humans. Data on the carcinogenicity of structural analogues do not indicate carcinogenic effects in the thyroid, although some produce liver tumors in mice; most analogues have not been studied for carcinogenicity. It is likely that compound 2 may pose a carcinogenic hazard to humans.

The existing mutagenicity information indicates negative effects for gene mutations in *Salmonella*. Some structural analogues produce structural chromosome aberrations in mammalian cells that could influence carcinogenicity.

There are data indicating that compound 2 affects thyroid-pituitary functioning. Within 90 days of dosing, there is thyroid enlargement associated with diffuse hyperplasia and an increase in thyroid weight. It is not known whether there is cellular hypertrophy in the thyroid. Hormone changes were investigated after dosing for only 14 days. Serum T₄ decreased with dosing while serum T₃ remained unchanged; TSH levels were not measured. There are no studies measuring hormones at times beyond 14 days.

Concerning the site of action, compound 2 produced liver enlargement in the chronic rat

study, and consistent with this, it induced microsomal enzymes in the rat liver (e.g., increased P-450 content and benzphetamine-N-demethylase activity) following a single, high acute dose. No studies have been conducted following subchronic dosing or at doses around those that produced thyroid tumors in rats.

A mode of action that accounts for production by compound 2 of thyroid tumors in rats can be proposed. It seems to be acting by nonmutagenic processes that involve chemically induced antithyroid activity. The compound may enhance metabolism and excretion of thyroid hormone by stimulating hepatic microsomal enzymes. These enzymes would be expected to enhance clearance of thyroid hormone from the body and result in a lowering of serum thyroid hormone levels. These decreases would result in reflex increases in TSH levels with stimulation and enlargement of the thyroid gland and, eventually, neoplasia. These observations and presumptions support the use of a default dose-response analysis using threshold considerations (use of an NOAEL and computation of the margins of exposure).

Significant uncertainties exist, which bring into question the proposed mode of action and the merit in using the margin of exposure method. Compound 2 has not been tested for cancer in a second species, and some related chemicals are known to induce liver tumors in mice. In addition, the agent has not been adequately tested for gene mutations, structural chromosome aberrations, and other such endpoints. The site of antithyroid action has not been adequately investigated; studies should investigate the thyroid and periphery as well as the liver where preliminary evidence suggests an effect. Mode-of-action studies are needed following subchronic dosing and at dosages adequate to explain thyroid tumor formation.

Because of the uncertainties and until such time that more information is available that explains a mode of action for compound 2, dose-response relationships will be projected by the low-dose linear approach.

There is not an extensive or totally appropriate database to evaluate the antithyroid effects of compound 2, but the existing dose correlations are summarized in table A-15, assuming that the liver may play some role in tumor development. Preneoplastic effects are noted at 15 mg/kg/day (thyroid weight) and 10 mg/kg/day (T_4 decrease), which are lower than the dose producing thyroid tumors (and increased liver weight) in the chronic study (50 mg/kg/day). An estimate of the NOAEL can be derived from the 14-day study where a dose of 5 mg/kg/day had no significant effect on the T_4 level. Further work is needed.

Depending on the outcome of future testing, a linear default may be maintained if the agent is mutagenic, if more than one tumor site is noted, or if adequate mode of action information is not developed to understand thyroid tumor production. A nonlinear default may be used if no other tumors are found in a second species, the compound is not mutagenic, and an antithyroid mode of action is found to describe the formation of thyroid tumors. Both methods

may be retained when more than one mode of action leads to the conclusion that both linear and nonlinear projections are about equally tenable.

BIBLIOGRAPHY

The following articles were consulted in developing the data used in this case study of the hypothetical chemical.

Comer, CP; Chengelis, CP; Levin, S; et al. (1985) Changes in thyroidal function and liver UDP-glucuronosyl-transferase activity in rats following administration of novel imidazole (SC-37211). *Toxicol Appl Pharmacol* 80:427-436.

Lumb, G; Newberne, P; Rust, JH; et al. (1978) Effects in animals of chronic administration of spironolactone—a review. *J Environ Pathol Toxicol* 1:641-660.

Sanders, JE; Eigenberg, DA; Bracht, LJ; et al. (1988) Thyroid and liver trophic changes in rats secondary to liver microsomal enzyme induction caused by an experimental leukotriene antagonist (L-649,923). *Toxicol Appl Pharmacol* 95:378-387.

Semler, DE; Chengelis, CP; Radzialowski, FM. (1989) The effects of chronic ingestion of spironolactone on serum thyrotropin and thyroid hormones in the male rat. *Toxicol Appl Pharmacol* 98:263-268.

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

EXECUTIVE SUMMARY

Compound 3 produces thyroid follicular cell and liver tumors in two rodent species. The thyroid tumors are associated with interference in thyroid-pituitary functioning, presumably due to inhibition of thyroid peroxidase activity. There is no mechanistic information concerning the development of liver tumors. Compound 3 is also DNA reactive and mutagenic, and these effects could influence tumor development in both the thyroid and the liver. Accordingly, it is likely that compound 3 may pose a human cancer hazard, and a low-dose linear procedure should be used for the quantification of both cancer dose-response relationships, although a lower bound on the thyroid cancer “risk” should also include nonlinear considerations.

DETAILED DATA

1. Cancer Findings

Chronic laboratory animal studies provide strong evidence of a tumorigenic effect of compound 3 for both the thyroid and liver in both sexes of rat and for the female mouse. F344 rats and B6C3F₁ mice were administered compound 3 at three doses of 200, 400, and 500 (20, 40, and 50 mg/kg/day) and 150, 300, and 800 ppm (30, 60, and 160 mg/kg/day) in tap water, respectively. There were 50 animals per sex/species/dose, with the control groups receiving plain tap water. No animal died before 52 weeks of dosing. Survival was lowest in the control male rats (50%) and the high-dose female rats (26%) at 104 weeks. In all other groups (rats and mice), survival ranged from 60% to 82%. The tumor incidence results are summarized in table 16 for both rats and mice.

The incidences of liver tumors (adenomas, carcinomas) were significantly increased in the low-, mid-, and high-dose male rats, in the mid- and high-dose female rats, and in the high-dose female mice. Male mice showed no increase in liver tumors. Although foci and areas of hepatocellular alteration were present, consistent with the preneoplastic phases of liver carcinogenesis, there was no indication of hepatocellular hypertrophy characteristic of MFO-inducing compounds.

The incidences of benign and malignant thyroid follicular cell neoplasms were significantly increased in the mid- and high-dose male and female rats and in the high-dose female mice (benign only). Male mice showed no increase in thyroid tumors. Tumors occurring at sites other than the liver and thyroid in rats and mice were not statistically related to treatment.

Table A-16. Rodents with liver and thyroid tumors in 2-year bioassays of compound 3

Rats	Tumor incidence (percent) according to dose (ppm in water) (N=50 animals/group)							
Sex	0		200		400		500	
	M	F	M	F	M	F	M	F
<u>Liver (hepatocellular)</u>								
Adenoma	1 (2)	3 (6)	9* (18)	0	18* (36)	20* (40)	17* (34)	11* (22)
Carcinoma	0	0	4* (8)	0	23* (46)	4* (8)	22* (44)	6* (12)
<u>Thyroid (follicular cell)</u>								
Adenoma	1 (2)	0	1 (2)	2 (4)	8* (16)	17* (34)	13* (26)	16* (32)
Carcinoma	0	0	2 (4)	2 (4)	9* (18)	12* (24)	15* (30)	7* (14)
Mice								
Sex	0		150		300		800	
	M	F	M	F	M	F	M	F
<u>Liver (hepatocellular)</u>								
Adenoma	11 (22)	4 (8)	13 (26)	6 (12)	11 (22)	9 (18)	10 (20)	14* (28)
Carcinoma	18 (36)	4 (8)	27 (54)	7 (14)	23 (46)	6 (12)	26 (52)	15* (30)
<u>Thyroid (follicular cell)</u>								
Adenoma	0	1 (2)	0	0	2 (4)	0	2 (4)	7* (14)

* $p < .05$ compared to control value.

2. Mechanistic Considerations

a. Mutagenicity

Compound 3 was studied in both in vitro and in vivo short-term tests for mutagenicity. In the *Salmonella* mutation assay, the compound was positive in the presence of an S9-activating system using strains TA-98 and TA-100. The compound induced transformation in BHK cells, DNA-strand breaks, and point mutations in V79 cells and DNA repair in rat hepatocytes. There was an equivocal response for sister chromatid exchanges in mouse bone marrow but positive results for chromosomal aberrations in the same system. Because compound 3 is DNA reactive and produces point and structural chromosomal mutations, it is regarded as having mutagenic activity relevant to carcinogenicity.

b. Thyroid Growth

To date, the only data generated for compound 3 specifically on thyroid weights were from a published account of its administration to male Wistar rats at 400 ppm in tap water for 20 weeks. At cessation of treatment, the mean values for thyroid weight were statistically significantly different in treated (30 ± 6 mg) as compared with control (18 ± 8 mg) groups (21 rats per group), respectively.

In the 13-week study, male and female Fischer rats were given 200, 400, and 700 ppm of compound 3 in tap water, while male and female mice received 100, 300, and 700 ppm. Thyroid follicular cell hyperplasia was found in 5/10 and 7/10 mid-dose male and female rats, respectively, but only in 1/9 high-dose males. Adenomatous goiters were observed in 3/10 mid-dose males and 1/10 mid-dose females, while more severe goiters were found at the high dose in 8/9 males and 10/10 females. In mice, adenomatous goiters (less severe than those found in rats) were observed in 1/10 of high-dose males and 1/10 high-dose females. None of the low-dose rats or mice showed thyroid lesions.

The incidence of diffuse hyperplasia of the thyroid gland in the 2-year bioassay was significantly increased in the mid- and high-dose rats of both sexes and the high-dose mice of both sexes, as summarized in table A-17.

In sum, many of these data indicate that compound 3 has an enhancing effect on thyroid growth by inducing diffuse thyroid follicular cell hyperplasia and adenomatous goiter and increased thyroid gland weight on repeated administration.

c. Hormone Levels

The studies completed so far provide limited data on the effect of compound 3 on serum levels of T_4 , T_3 , and TSH. Serum levels for these three hormones were measured in a group of

Table A-17. Rats and mice with diffuse thyroid hyperplasia after 2 years of exposure to compound 3

Incidence (percent) according to dose (ppm in water) (N=50 animals/group)									
Rats	Sex	0		200		400		500	
		M	F	M	F	M	F	M	F
		0	0	1 (2)	1 (2)	11* 16* (22)	(32)	13* (26)	22 (44)
Mice	Sex	0		100		300		700	
		M	F	M	F	M	F	M	F
		0	0	0	0	0	0	26* 25* (52)	(50)

* $p < .05$ compared to control value.

five male rats after exposure to 400 ppm of compound 3 for 20 weeks and compared with those from a control group (also five male rats), as illustrated in table A-18. Circulating levels of both T_3 and T_4 were significantly reduced and TSH was significantly elevated, indicating an effect of the compound on thyroid-pituitary functioning.

d. Site of Action

No studies have yet been conducted to investigate the action of compound 3 on components of the iodide pump, thyroid peroxidase, peripheral deiodination of T_4 , or MFO induction. However, some close analogues are known to reduce radioactive iodine accumulation in the thyroid; presumably this is due to an inhibition of thyroid peroxidase activity and not a function of affecting the iodide pump.

In the animal bioassays, the liver shows sinusoidal dilatation in male rats, fatty metamorphosis and focal cellular change in male and female rats, and degeneration in male mice. It has been speculated that the metabolic activation of compound 3 to a reactive electrophile may be responsible for these nonneoplastic lesions. This is consistent with the *Salmonella* mutation data, where effects are noted only after metabolic activation. No other information is available concerning the biological processes leading to liver tumor development.

e. Dose Correlations

Important correlations can be made between the dose of compound 3 and various effects. These are discussed later in this appendix (see table A-19).

Table A-18. Serum T₄, T₃, and TSH levels in male Wistar rats exposed to 400 ppm of compound 3 in water for 20 weeks

	Control	Treated
T ₃ (ng/dL)	54.6±1.8	40.2±2.2*
T ₄ (µg/dL)	5.2±1.1	3.0±0.3*
TSH (ng/mL)	4.1±0.8	6.1±0.7*

**p*<.05 compared to control value.

Table A-19. Thyroid effects noted in the toxicity studies in rats and mice on compound 3

	Dose in drinking water (ppm)				
	200	400	500	700	
Rats					
2-Year study					
Tumors	-	+	+		
Hyperplasia	-	+	+		
13-Week study					
Hyperplasia/goiter	-	+		+	
20-Week study					
Thyroid/pituitary hormones and thyroid weight		+			
Mice	100	150	300	700	800
2-Year study					
Tumors		-	-		+
Hyperplasia		-	-		+
13-Week study					
Goiter	-		-	+	

f. Metabolic and Pharmacokinetic Properties

No information is available on the tissue distribution of compound 3. It has been estimated that 90% is absorbed through the gastrointestinal and respiratory tracts in rats. The compound is rapidly excreted in the urine with two major metabolites. No comparative metabolic information is available in mice or humans.

g. Structure-Activity Relationships

Compound 3 is a member of the bis-benzenamine class of chemicals, being an alkyl-substituted derivative of 4,4'-methylene-dianiline. Several dianilines with methylene, oxygen and sulfur bridges, and their alkyl-substituted derivatives have been shown to (1) inhibit iodine accumulation in the thyroid, (2) possess goitrogenic activity following repeated administration, and (3) produce thyroid tumors and sometimes liver tumors in rats and/or mice. These aromatic amines are mutagenic in a number of short-term tests relevant for evaluation of carcinogenicity, namely, DNA reactivity and gene and structural chromosome mutations.

3. Human Observations

No data are available concerning the effects on humans of exposure to compound 3. However, it is known from accidental and certain high-dose industrial incidents that exposure to structurally related chemicals in the bis-benzenamine class have the potential to produce toxic hepatitis.

HAZARD AND DOSE-RESPONSE CHARACTERIZATION

Compound 3 is likely to pose a carcinogenic hazard to humans given that it induces both thyroid and liver tumors in rodents and has gene mutagenic activity in microbes and cultured mammalian cells. Mutagenic properties may account for the tumor responses; in addition, the thyroid tumors may be due to perturbation in thyroid-pituitary functioning. Therefore, cancer dose-response relationships should be projected using a low-dose linear procedure; a lower bound on the thyroid cancer risks may be characterized using nonlinear considerations.

Cancer studies on the bis-benzenamine, compound 3, show significant increases in thyroid follicular cell carcinomas and adenomas in male and female rats and adenomas alone in female mice; male and female rats and female mice also show significant increases in hepatocellular carcinomas and adenomas. Male mice do not show increases in either of these tumor types. Structurally related compounds often produce thyroid tumors in rats and mice and liver tumors in mice. Just as compound 3 shows DNA reactivity, gene mutations, and possible structural chromosome mutations, structural analogues also produce mutagenic effects relevant to carcinogenicity.

Several lines of investigation indicate that compound 3 affects thyroid-pituitary functioning. (1) Significant dose-related goitrogenic effects (e.g., increase in thyroid weight, diffuse hyperplasia) are noted after subchronic and chronic dosing in rats and mice. There is no mention whether follicular cell hypertrophy was noted in any of the studies. (2) Following 20 weeks of treatment, hormone levels are altered in rats, including decreases in both T_4 and T_3 and increases in TSH. (3) No studies have been done on compound 3 to discern its site of action on thyroid-pituitary functioning, although several structural analogues inhibit the accumulation of iodide in the rat thyroid, probably the result of thyroid peroxidase inhibition. (4) Some indication of progression of histologic lesions is noted: thyroid hyperplasia and/or adenomatous goiter develop after subchronic dosing in rats and mice, while neoplasms form after chronic dosing. In mice, seemingly the more resistant species (on a mg/kg/day basis), the only thyroid neoplasms are adenomas, while in rats, both adenomas and carcinomas are found in roughly comparable frequencies. (5) Dose correlations are presented in table A-19 and are discussed later. In sum, these observations constitute an adequate database to evaluate antithyroid effects, but there is a lack of specific information on the site of action.

The above influences of compound 3 on thyroid-pituitary status could account for thyroid tumor formation following chronic exposure. However, there is also mutagenic activity relevant to carcinogenicity for both compound 3 and structural analogues that include DNA reactivity, gene mutations, and structural chromosome aberrations. The compound is seemingly metabolized to produce reactive products that are mutagenic. Only minimal metabolic information is available in rats, and none exists in mice and humans. These mutagenic effects could account for both the thyroid and liver tumors.

Characterization of cancer dose-response relationships should primarily rely on mutagenic considerations for the thyroid and liver tumors using a low-dose linear procedure. However, the thyroid tumor responses may be due to both its mutagenic and antithyroid properties. Other chemicals with both mutagenic and antithyroid effects also have led to high thyroid tumor incidences, as have combinations of mutagenic and antithyroid stimuli. Because it is not possible to totally discern the relative impacts of these influences for compound 3, threshold considerations should be used in addition to a linear extrapolation so as to estimate the lower bound on the thyroid cancer risk.

In making a decision about potential thyroid cancer risk due to antithyroid activity, toxicity dose correlations on compound 3 are important considerations (table A-19). Thyroid effects are noted in rats at doses of 400 ppm and higher in studies spanning the subchronic and chronic dosing periods, but not at 200 ppm. In mice, effects are noted at 700 and 800 ppm, but not at 300 ppm and below. It is recognized that there are thyroid-pituitary hormone levels only in rats after dosing with 400 ppm and no data in mice; more extensive testing in mice would help to

ensure identification of doses where hormone alterations are not noted. In their absence, emphasis can be placed on the above identified no-observed-effect-levels (NOEL), recognizing that there is some degree of uncertainty in relying on the values. Rats appear to be as sensitive or more sensitive to the antithyroid effects of compound 3 as mice on a mg/kg/day basis. With the existing database, the estimated NOEL for rats is 200 ppm (20 mg/kg/day), while that for mice is 300 ppm (60 mg/kg/day). The 20 mg/kg/day value in rats can be used in calculating margins of exposure. Linear risks for thyroid tumors might be estimated by drawing a straight line from the 10% estimated tumor incidence level to the origin or the lower 95% bound on that incidence. Risks for other tumors would depend upon the mode of action information available on those sites.

BIBLIOGRAPHY

The following articles were consulted in developing the data used in the case study of this hypothetical chemical.

McQueen, CA; Williams, GM. (1990) Review of the genotoxicity and carcinogenicity of 4,4'-methylene-dianiline and 4,4'-methylene-bis-2-chloroaniline. *Mutat Res* 239:133-142.

Weisberger, EK; Murthy, ASK; Lilja, HS; et al. (1984) Neoplastic response of F344 rats and B6C3F₁ mice to the polymer and dyestuff intermediates 4,4'-methylenebis(N,N-dimethyl)benzenamine, 4,4'-oxydianiline, and 4,4'-methylenedianiline. *J Natl Cancer Inst* 72:1457-1463.

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

EXECUTIVE SUMMARY

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 golden hamsters. Compound 4 is mutagenic in various short-term tests for DNA alkylation, DNA damage, and gene and structural chromosome mutations. Because compound 4 is mutagenic, causes tumors with short latencies at multiple anatomical sites including the thyroid, and does not appear to interrupt the thyroid endocrine axis, the thyroid follicular cell tumors appear to be caused by a mutagenic mechanism, indicating that linear risk assessment procedures for low-dose extrapolation apply in this case.

DETAILED DATA

1. Cancer Findings

Although compound 4 has not been tested in a conventional 2-year rodent bioassay, tumor incidence data for the chemical are available from various shorter term biomedical research studies. The results from one of these studies using male Wistar rats are summarized in table A-20. The doses administered to groups of 20 rats were 0, 50, 100, and 200 ppm (corresponding to 0, 5, 10, and 20 mg/kg/day or 0, 910, 1,820, and 3,640 mg/kg total dose) in drinking water that was provided ad libitum. The study was terminated after 26 weeks of exposure. Although only one death (in the high-dose group) had occurred at that time, adenomas and carcinomas of the lung, liver, kidneys, and thyroid were found. Lung tumors, induced at all dose levels tested, were found in 100% of the mid- and high-dose animals. Tumors in the liver, kidney, and thyroid occurred with lower incidences at the mid- and high-dose levels, but showed dose responsiveness.

Separate studies have shown that compound 4 is effective in producing tumors in a similar range of organ sites by other routes of administration, for example, by weekly subcutaneous injection in both male and female Sprague-Dawley rats or by i.p. injection to Wistar rats. Another study, using oral administration, demonstrated compound 4's ability to produce pancreatic cancer, as well as lung and liver tumors, in the Syrian golden hamster. In the experiment using the i.p. route of administration, compound 4 was administered to male and female rats at three dose levels once weekly for 4 weeks. The experiment was

Table A-20. Wistar rats with tumors by site in a 26-week study of compound 4

Tumor incidence (percent) according to dose				
Organ site	0	50	100	200
Lung	0/20	5/20 (25)	20/20 (100)	19/19 (100)
Liver	0/20	0/20	5/20 (25)	8/19 (42)
Kidney	0/20	0/20	3/20 (15)	4/19 (21)
Thyroid	0/20	0/20	0/20 (10)	5/19 (26)

terminated at 30 weeks, at which time thyroid tumors were diagnosed at the mid and high doses in both male and female rats (table A-21). There was a particularly high incidence (80%) in the high-dose males. Since the cumulative doses in the i.p. study were well below those in the drinking water study, the i.p. route appears to be a more effective mode for inducing thyroid tumors in the rat with compound 4.

These data in both sexes of multiple species, multiple organs, and by different routes of administration indicate that compound 4 is a potent carcinogen in laboratory animals. The chemical's potency is further emphasized by the very short latency period of induction, the low total dose of the chemical, and the high tumor incidences.

2. Mechanistic Considerations

a. Mutagenicity

A review of the literature indicates that compound 4 is consistently positive in short-term tests for mutagenicity in the presence of a metabolic activation system. It has tested positively in various *Salmonella* assays for frameshift and base-pair mutations with strains TA-1537 and TA-1535, respectively, and produces sister chromatid exchange and chromosomal aberrations in CHO cells and transformation of BHK cells. DNA reactivity has been shown by positive results for DNA single-strand breakage in cultured mouse epithelial cells, for alkylation both in vitro and in vivo, and for inducing DNA-repair replication in human lymphocytes.

Table A-21. Thyroid follicular cell tumor incidence at 30 weeks in male and female Wistar rats treated with compound 4 by i.p. injection

Total cumulative dose (mg/kg)				
	0	200	400	600
Male	0	1/25 (4)	6/25 (24)	20/25 (80)
Female	0	0	1/24 (4)	5/24 (21)

b. Thyroid Growth

The effects of compound 4 on the thyroid, both grossly and histologically, were different from the effects of chemicals acting on the thyroid-pituitary axis. Thyroid weights were recorded at 30 weeks in the i.p. injection cancer study of compound 4. These are summarized in table A-22.

Although thyroid follicular cell tumors were present in the mid- and high-dose males and in the high-dose females, thyroid weights overall showed no statistically significant difference among groups and therefore no correlation with the incidences of thyroid tumors. This lack of statistical significance remained when the thyroid weights were calculated relative to body weights. At the histologic level, the thyroids from treated groups showed a dose-response relationship for the incidence of follicular cell hyperplasia (table A-23). The hyperplasia, however, was not of the diffuse form typical of antithyroid compounds, but manifested as small solitary foci, presumably representing the first stage in the continuum of hyperplasia to adenoma to carcinoma. Thus, focal hyperplasias were described as small aggregates of basophilic epithelial cells involving part of a follicle or a few follicles, but without fibrous encapsulation. Uninvolved tissue lacked cellular hypertrophy, and the follicles contained amounts of colloid within the normal range.

c. Hormone Levels

In the i.p. cancer study, serum T₄ and TSH levels (but not T₃) were also measured (summarized in table A-24). There were no differences in serum T₄ or TSH levels in treated groups when compared with levels in control animals. In addition, comparison of serum TSH concentrations in high-dose rats with and without thyroid tumors demonstrated no significant differences (table A-25).

Table A-22. Mean thyroid weights (mg) at 30 weeks in male and female Wistar rats treated with compound 4 by i.p. injection

Total cumulative dose (mg/kg)				
	0	200	400	600
Male	15.6±2.2	15.2±2.2	14.8±1.4	29.9±35.5*
Female	11.1±2.6	10.6±1.3	10.8±1.3	11.9±3.5

*The large variation in thyroid weights in the high-dose males was due to the presence of several very large tumors. The high standard deviation for this group resulted in a lack of significance from controls.

Table A-23: Incidence of focal hyperplasia involving thyroid follicular cells at 30 weeks in male and female Wistar rats treated i.p. with compound 4

Total cumulative dose (mg/kg)				
	0	200	400	600
Male	0	4/25 (16)	9/25 (36)	18/25* (72)
Female	0	0	1/24 (4)	3/24 (12)

* $p < .05$ compared with control values.

Table A-24. Serum T₄ and TSH levels at 30 weeks in male and female Wistar rats treated i.p. with compound 4

Total cumulative dose (mg/kg)								
	0		200		400		600	
	M	F	M	F	M	F	M	F
T ₄ (ug/dl)	3.4 ±0.5	2.9 ±0.7	3.8 ±0.4	3.3 ±0.4	3.0 ±0.5	2.3 ±0.7	3.3 ±0.4	3.0 ±0.6
TSH (ng/ml)	4.5 ±1.1	3.2 ±0.5	5.8 ±2.5	3.9 ±1.4	4.0 ±1.0	4.4 ±1.5	4.4 ±1.1	4.0 ±1.2

Table A-25. Comparison of serum TSH levels in rats with and without thyroid tumors induced by i.p. treatment with a cumulative dose of 600 mg/kg of compound 4

	No. of rats		TSH concentration (ng/ml)	
	M	F	M	F
Without tumors	9	19	4.9±1.4	4.0±1.1
With tumors	11	3	4.2±1.0	4.0±0.5

A separate study provided limited information on serum T₃ levels at 20 weeks posttreatment in male Wistar rats receiving a single i.p. injection of compound 4 at a dose of 1,000 mg/kg. Compared to the mean value of 80.8±3.8 ng/ml for 20 control animals, the mean value for serum T₃ in the treated group (n=20) was not significantly different at 90.7±11.5 ng/ml.

These various data, showing serum T₃, T₄, and TSH levels within the control ranges after treatment with compound 4, provide further evidence that the chemical has no effect on the function of the thyroid/pituitary axis.

d. Site of Action

No studies were available that have investigated the effects of compound 4 on thyroid peroxidase, the deiodination pathway, or effects on thyroid metabolism and excretion in the liver.

However, the chemical is without goitrogenic or thyroid-pituitary hormone effects, indicating that such studies are not needed. Obviously, there are no significant dose correlations to consider.

e. Ancillary Data

The initiating effect of compound 4 on two-stage thyroid carcinogenesis was investigated using the antithyroid agent propylthiouracil (PTU) as a promoter. Male Wistar rats, 6 weeks old, were administered a single 1,000 mg/kg i.p. dose of compound 4 and fed PTU in the basal diet for 19 weeks (beginning of week 2 to week 20) at a level of 0.15%. The experiment was terminated at 20 weeks, and the thyroids were examined histologically for neoplastic lesions. The results are tabulated in table A-26. The incidence of thyroid follicular tumors in the group receiving compound 4 followed by administration of PTU was 95% compared to 5% in the compound 4 only group and 0% in the PTU only group. The results provide evidence that compound 4 acts as an initiating agent for the development of thyroid tumors in rats that can be promoted with PTU.

Table A-26. Male rats with thyroid tumors at 20 weeks after a single treatment with compound 4 followed by PTU

	Treatments*			
	None	Compound 4	PTU	Compound 4 + PTU
No. (%) of rats with thyroid tumors	0/20 (0)	1/20 (5)	0/20 (0)	19/20 (95)

* Compound 4: single i.p., 1,000 mg/kg; PTU: 0.15% in diet for 19 weeks.

f. Structure-Activity Relationships

Compound 4, a nitrosamine, belongs to a notorious class of chemicals recognized to be potent mutagenic carcinogens, producing tumors in many anatomical sites in both sexes of multiple species. Many other members of this class are also used in research studies involving mechanisms of carcinogenesis. Enzymic hydroxylation of these chemicals is required for generation of the proximate carcinogens/mutagens, which are strong alkylating agents.

g. Metabolism and Pharmacokinetic Properties

It is known from mechanistic studies that compound 4 is rapidly and evenly distributed throughout the body water. Although approximately 60% of the administered dose is excreted unchanged in the urine, the chemical is also oxidized in the liver to a ketone that has been shown to be a methylating agent. The enzyme system responsible for oxidation has not been determined.

3. Human Data

No direct information exists on the acute or chronic effects of this chemical in humans.

HAZARD AND DOSE-RESPONSE CHARACTERIZATION

Compound 4 produces tumors at several sites, including the thyroid. The thyroid tumors do not result from disruption of thyroid-pituitary status. The chemical does not produce follicular cell hypertrophy or diffuse hyperplasia and does not alter the levels of circulating thyroid hormones or TSH. Although there are no specific studies on the chemical's effect on thyroid hormone synthesis or disposition, there is no indication that it has such effects. Instead, it appears to act, as do many other members of the nitrosamine group, as a clear-cut mutagenic carcinogen. It possesses demonstrable gene and chromosome-breaking mutagenic activity and an ability to alkylate DNA both in vitro and in vivo. It also causes DNA damage, has initiating capacity in a thyroid two-stage carcinogenesis test, acts as a complete carcinogen producing tumors at multiple sites in addition to the thyroid, is effective as a carcinogen by more than one route of administration and in more than one species, and exhibits a very short tumor latency. For these reasons, compound 4 has the potential to be a human carcinogen, functioning as a mutagenic carcinogen rather than by disruption of thyroid-pituitary status. Accordingly, thyroid dose-response assessments for this substance should be conducted using a low-dose linear procedure. Risk estimation at other sites would depend on the mode of action information.

BIBLIOGRAPHY

The following articles were consulted in developing the data used in this case study of this hypothetical chemical.

Hiasa, Y; Kitahori, Y; Kato, Y; et al. (1987) Potassium perchlorate, potassium iodide, and propylthiouracil: promoting effect on the development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)nitrosamine. *Jpn J Cancer Res* 78:1335-1340.

Hiasa, Y; Kitahori, Y; Kitamura, M; et al. (1991) Relationships between serum thyroid stimulating hormone levels and development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 12:873-877.

Kitahori, Y; Hiasa, Y; Konishi, N; et al. (1984) Effect of propylthiouracil on the thyroid tumorigenesis induced by N-bis(2-hydroxypropyl)nitrosamine in rats. *Carcinogenesis* 5:657-660.

Kitahori, Y; Hiasa, Y; Katoh, Y; et al. (1988) Promotive effect of 4,4'-methylenebis(N,N-dimethyl)benzeneamine on N-bis(2-hydroxypropyl)nitrosamine-induced thyroid tumors in Wistar rats. *Cancer Lett* 40:275-281.

Konishi, Y; Denda, A; Kondo, H; et al. (1976) Lung carcinomas induced by oral administration of N-bis(2-hydroxypropyl)nitrosamine in rats. *Gann* 67:773-780.

Konishi, Y; Kondo, H; Ikeda, T; et al. (1987) Effect of dose on the carcinogenic activity of orally administered N-bis(2-hydroxypropyl)nitrosamine in rats. *Gann* 69:573-577.

Mohr, U; Reznik, G; Pour, P. (1977) Carcinogenic effects of diisopropanolnitrosamine in Sprague-Dawley rats. *J Natl Cancer Inst* 58:361-366.

APPENDIX B

SYNOPSIS OF AGENTS AFFECTING THE THYROID

APPENDIX B

SYNOPSIS OF AGENTS AFFECTING THE THYROID

Extensive investigation of physical and chemical factors have identified many treatments that influence the thyroid gland. Some directly affect thyroid gland functioning (table B-1), such as those that damage the genetic material and those that in some way affect the synthesis or release of thyroid hormone from the gland. Other agents influence the thyroid indirectly (table B-2), as by reducing the formation of circulating T_3 from T_4 due to inhibition of 5'-monodeiodinase. Chemicals from many different structural and functional classes also indirectly affect the thyroid by inducing liver microsomal enzymes and increasing the metabolism and excretion of thyroid hormones.

Still other agents can affect the thyroid in more than one way; several examples are applicable. Thiocyanate ion inhibits both iodide uptake into the thyroid and thyroid peroxidase activity, while propylthiouracil inhibits both thyroid peroxidase and 5'-monodeiodinase activities. These agents doubly reduce effective thyroid hormone levels. Other compounds may have both thyroid cancer initiation and promotion activity. For instance, both 3-methylcholanthrene and 4,4'-methylenedianiline are mutagenic and may affect thyroid cell DNA, while the former is also a liver microsomal enzyme inducer that increases thyroid hormone metabolism and excretion and the latter also reduces thyroid hormone synthesis by probably inhibiting thyroid peroxidase.

Not all agents that have some direct or indirect effect on the thyroid have demonstrated carcinogenic effects. In humans, for instance, only x-irradiation has been demonstrated to produce thyroid cancer. In rodents, many compounds that have produced some effect on the thyroid have never been tested in chronic studies. Even those that have been tested do not always produce thyroid tumors. For instance, phenytoin produces many different effects on the thyroid, but it was negative for thyroid tumors in rodent studies conducted by the National Toxicology Program (NTP). The same is true for certain polyhydroxy aromatic compounds that are inhibitors of thyroid peroxidase, in that neither resorcinol nor 4-hexylresorcinol produced thyroid tumors in NTP rodent studies. This is interesting, given that resorcinol has been shown to be goitrogenic in short-term animal studies and in humans.

Liver microsomal enzyme inducers are very heterogeneous: they span many different structural and functional classes of chemicals, they differ in regard to the specific microsomal enzymes that are induced, and they vary greatly in their potency for enzyme induction.

Table B-1. Illustrative treatment regimens directly affecting the thyroid gland

<u>Iodide deficiency</u>	<u>Thyroid peroxidase inhibition</u>
	thionamides
<u>Iodide pump inhibition</u>	goitrin
perchlorate ion	propylthiouracil
pertechnetate ion	thiocyanate ion
thiocyanate ion	thiouracil
	aniline derivatives
<u>Thyroid hormone release inhibition</u>	4,4'-methylenedianiline
excess iodide	p-aminobenzoic acid
lithium	sulfamethazine
	sulfathiazole
<u>Thyroid gland damage</u>	polyhydroxy aromatics
polybrominated biphenyls	4-hexylresorcinol
polychlorinated biphenyls	phloroglucinol
	resorcinol
	miscellaneous
	2-aminothiazole
	amitrole
	<u>Mutagenic</u>
	x-irradiation
	¹³¹ I
	glycidol
	3-methylcholanthrene
	4,4'-methylenedianiline
	N-bis (2-hydroxypropyl) nitrosamine
	tribromomethane

Accordingly, only some enzyme inducers have been shown to influence thyroid-pituitary functioning following short-term administration. Among those with antithyroid effects, thyroid-pituitary status may return to homeostasis following continued dosing. Thus, in long-term dosing studies in rodents, only a proportion of these agents go on to induce thyroid neoplasia. Generally, thyroid carcinogenic responses are noted in a relatively low percentage of animals with the enzyme inducers, in contrast to those agents that inhibit thyroid peroxidase that often produce much more pronounced carcinogenic effects.

As of February 1996, the experience from the NTP indicates that 33 of approximately 460 chemicals (about 7%) (table B-3) tested in chronic studies show thyroid follicular tumors in rats and/or mice. A number of others show some preneoplastic lesion (usually hyperplasia but

Table B-2. Illustrative agents indirectly influencing the thyroid gland

<u>Liver microsomal enzyme induction</u>	<u>5'-Monodeiodinase inhibition</u>
channel blocker	amiodarone
nicardipine	FD&C Red No. 3
CNS active	iopanoic acid
phenobarbital	propranolol
phenytoin	propylthiouracil
histamine (H ₂) antagonist	
SK&F 93479	
CoA reductase inhibitor	
simvastatin	
imidazole antibiotic	
SC-37211	
leukotriene antagonist	
L-649,923	
pesticide	
clofentezine	
thiazopyr	
polyaromatic hydrocarbon	
3-methylcholanthrene	
polyhalogenated hydrocarbon	
C ₁₂ chlorinated paraffins	
dieldrin	
polychlorinated biphenyls	
2,3,7,8-tetrachlorodibenzo-p-dioxin	
toxaphene	
retinoid	
etretinate	
steroid	
spironolactone	

also hypertrophy) without corresponding neoplasia. Most of the tested chemicals have not been studied as to their antithyroid potential. However, it appears that a number of these chemicals inhibit thyroid peroxidase or are microsomal enzyme inducers; still others have mutagenic activity that might account for the thyroid neoplasms. For instance, short-term toxicity studies have been completed on the thionamide 2-mercaptobenzimidazole that show both significant thyroid hyperplasia (Gaworski et al., 1991) and antithyroid activity; thyroid tumors would be expected in chronic studies. Interestingly, 2-mercaptobenzothiazole, a closely related compound, failed to show antithyroid effects (Bywater et al., 1945) and likewise failed to induce thyroid cancer in NTP chronic testing.

Table B-3. Chemicals producing thyroid follicular cell tumors in NTP studies

aldrin	isobutyl nitrite
3-amino-4-ethoxyacetanilide	malonaldehyde, sodium salt
o-anisidine hydrochloride	manganese sulfate monohydrate
azinphosmethyl	mercuric chloride
2,2-bis (bromomethyl)-1,3-propanediol	4,4'-methylenebis (N,N-dimethyl) benzenamine
tertiary butyl alcohol	4,4'-methylenedianiline dihydrochloride
chlorinated paraffins: C12, 60% chlorine	1,5-naphthalenediamine
C.I. Basic Red 9 monohydrochloride	oxazepam
C.I. Pigment Red 3	4,4'-oxydianiline
decabromodiphenyl oxide	2,3,7,8-tetrachlorodibenzo-p-dioxin
2,4-diaminoanisole sulfate	p,p'-tetrachlorodiphenylethane (DDD)
N,N'-diethylthiourea	1-trans-delta-9-tetrahydrocannabinol
ethylene thiourea (ETU)	4,4'-thiodianiline
glycidol	toxaphene
HC Blue No. 1	trimethylthiourea
heptachlor	tris (2-chloroethyl) phosphate
iodinated glycerol	

REFERENCES

Bywater, WG; McGinty, DA; Jenesel, ND. (1945) Antithyroid studies. II. The goitrogenic activity of some imidazoles and benzimidazoles. *J Pharmacol Exp Therap* 85:14-22.

Gaworski, CL; Aranyi, C; Vana, S; et al. (1991) Prechronic inhalation toxicity studies of 2-mercaptobenzimidazole (2-MB) in F344/N rats. *Fundam Appl Toxicol* 16:161-171.

APPENDIX C

**SCIENTIFIC FINDINGS FROM
1988 EPA REVIEW DOCUMENT**

REVIEW

Thyroid Follicular Cell Carcinogenesis¹

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Thyroid Follicular Cell Carcinogenesis. HILL, R. N., ERDREICH, L. S., PAYNTER, O. E., ROBERTS, P. A., ROSENTHAL, S. L., AND WILKINSON, C. F. (1989). *Fundam. Appl. Toxicol.* 12, 629-697. Ample information in experimental animals indicates a relationship between inhibition of thyroid-pituitary homeostasis and the developmental thyroid follicular cell neoplasms. This is generally the case when there are long-term reductions in circulating thyroid hormones which have triggered increases in circulating thyroid stimulating hormone. Such hormonal derangements leading to neoplasms have been produced by different regimens, including dietary iodide deficiency, subtotal thyroidectomy, and administration of natural and xenobiotic chemical substances. The carcinogenic process proceeds through a number of stages, including follicular cell hypertrophy, hyperplasia, and benign and sometimes malignant neoplasms. Given the interrelationship between the thyroid and pituitary glands, conditions that result in stimulation of the thyroid can also result in stimulation of the pituitary, with the development of hyperplastic and neoplastic changes. The progression of events leading to thyroid (and pituitary) neoplasms can be reversed under certain circumstances by reestablishing thyroid-pituitary homeostasis. Most chemicals that have induced follicular cell tumors seem to operate through inhibition of the synthesis of thyroid hormone or an increase in their degradation and removal. For some of these compounds, it appears that genotoxic reactions may not be playing a dominant role in the carcinogenic process. A seemingly small group of thyroid carcinogens seems to lack influence on thyroid-pituitary status and may in part be operating via their genotoxic potential. In contrast with the well-established relationship between thyroid-pituitary derangement and follicular cell neoplasms in animals, the state of information in humans is much less certain. At this time, ionizing radiation is the only acknowledged human thyroid carcinogen, a finding well established in experimental systems as well. Although humans respond to goitrogenic stimuli as do animals, with the development of cellular hypertrophy, hyperplasia, and under certain circumstances nodular lesions, disagreement exists as to whether malignant transformation occurs in any predictable manner. It would seem that if humans develop thyroid tumors following long-term derangement in thyroid-pituitary status, they may be less sensitive than the commonly used animal models.

¹ This document has been reviewed in accordance with the U.S. Environmental Protection Agency procedures and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does the mention of trade names or commercial products

constitute endorsement or recommendation for use.

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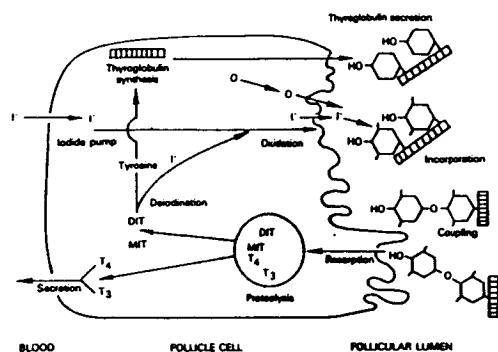


FIG. 1. Schematic representation of thyroid hormone biosynthesis and secretion. The protein portion of thyroglobulin is synthesized on rough endoplasmic reticulum, and carbohydrate moieties are added by the Golgi apparatus. Thyroglobulin proceeds to the apical surface in secretory vesicles which fuse with the cell membrane and discharge their contents into the lumen. Iodide enters the cell by active transport, is oxidized by a peroxidase at the apical border, and is incorporated into tyrosine residues in peptide linkage in thyroglobulin. Two iodinated tyrosyl groups couple in ether linkage to form thyroxine, which is still trapped in thyroglobulin. For the secretory process, thyroglobulin is engulfed by pseudopods at the apical border of the follicular lumen and resolved into vesicles that fuse with lysosomes. Lysosomal protease breaks down thyroglobulin to amino acids, T_4 , T_3 , diiodotyrosine (DIT), and monoiodotyrosine (MIT). T_4 and T_3 are secreted by the cell into the blood. DIT and MIT are deiodinated to free tyrosine and iodide, both of which are recycled back into iodinated thyroglobulin. Source: Goodman and van Middlesworth (1980).

I. THYROID-PITUITARY PHYSIOLOGY AND BIOCHEMISTRY

In order to examine the possible role of pituitary, thyroid, and related hormones in thyroid carcinogenesis, it is important to first understand the physiology and biochemistry of the thyroid-pituitary hormonal system. Accordingly, this section summarizes the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated. References are mainly to recent reviews (see Paynter *et al.*, 1986; 1988) rather than to the original scientific literature.

A. Synthesis of Thyroid Hormones

The thyroid hormones are synthesized in the thyroid gland and are stored as amino acid residues of thyroglobulin, a protein constituting most of the colloid in the thyroid fol-

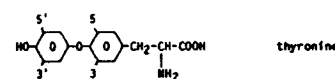
licles (Goodman and van Middlesworth, 1980; Taurog, 1979; Haynes and Murad, 1985). Thyroglobulin is a complex glycoprotein made up of two identical subunits each with a molecular weight of 330,000 D.

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

The next step in the process is a concerted reaction in which iodide is oxidized to an active iodine species that in turn iodates the



Monoiodotyrosine (MIT) = 3-iodotyrosine
 Diiodotyrosine (DIT) = 3,5-diiodotyrosine



Thyroxine (T_4) = 3,5,3',5'-tetraiodothyronine
 Triiodothyronine (T_3) = 3,5,3'-triiodothyronine
 Reverse triiodothyronine (rT_3) = 3,3',5'-triiodothyronine

FIG. 2. Iodinated compounds of the thyroid gland.

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

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

hydrolyzed completely to permit release of T_4 and T_3 .

Although T_4 is by far the major thyroid hormone secreted by the thyroid (normally about 8 to 10 times the rate of T_3 , although it varies as a function of the iodine intake), it is usually considered to be a prohormone. Thus, T_3 is about fourfold more potent than T_4 , and about 33% of the T_4 secreted undergoes 5'-deiodination to T_3 in the peripheral tissues; another 40% undergoes deiodination of the inner ring to yield the inactive material, reverse triiodothyronine (rT_3) (Fig. 2).

B. Transport of Thyroid Hormones in Blood

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

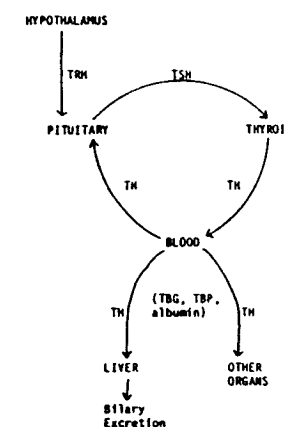


FIG. 3. Hypothalamic-pituitary-thyroid-peripheral organ relationships. TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TH, thyroid hormones; TBG, thyroxine-binding globulin; TBP, thyroxine-binding prealbumin.

and rabbits (Dohler *et al.*, 1979). Thyroxine-binding prealbumin and albumin also transport thyroid hormones in the blood; the prealbumin has K_a values of about 10^7 and 10^6 M for T_4 and T_3 , respectively. Normally, only about 0.03% of the T_4 in the circulation is free and available for cell membrane penetration and thus hormone action, metabolism, or excretion. The levels of free thyroid hormones in the circulation may be changed through competitive binding interactions of certain drugs and other foreign compounds (Haynes and Murad, 1985).

C. Metabolism and Excretion

As previously discussed, T_4 , the major hormone secreted from the thyroid, is considered to be a prohormone and is converted to the more active T_3 by a 5'-monodeiodinase in a variety of peripheral tissues, including the pituitary. T_4 is also metabolized to rT_3 which is hormonally inactive and has no known function, except perhaps as an inhibitor of the conversion of T_4 to T_3 . The 5'-monodeiodinase also reacts with rT_3 and converts it to a diiodo-derivative (Larson, 1982b). Under normal conditions the half-life of T_4 is 6 to 7 days in humans (see Thomas and Bell, 1982).

Degradative metabolism of the thyroid hormones occurs primarily in the liver and involves conjugation with either glucuronic acid (mainly T_4) or sulfate (mainly T_3) through the phenolic hydroxyl group. The resulting conjugates are excreted in the bile into the intestine. A portion of the conjugated material is hydrolyzed in the intestine, and the free hormones thus released are reabsorbed into the blood (enterohepatic circulation). The remaining portion of the conjugated material (20 to 40% in humans) is excreted in the feces.

As stated previously, most thyroid hormone is carried in the blood of humans by thyroxine-binding globulin and thyroxine-binding prealbumin. In the absence of thyroxine-binding globulin, as in the rat and mouse, more thyroid hormone is free of pro-

tein binding and is subject to metabolism and removal from the body. As a consequence, the half-life of T_4 in the rat is only about 12-24 hr in contrast to 6-7 days in humans (see Thomas and Bell, 1982). To compensate for the increased turnover of thyroid hormone, the rat pituitary secretes more TSH. Baseline serum TSH levels in humans are on the order of 2.5 μ U/ml, while in rats it ranges from 55.5 to 65 μ U/ml in males and 36.5 to 41 μ U/ml in females, (about a 2-fold sex difference). It has been suggested that rats require a 10-fold higher T_4 production rate per kilogram body weight than do humans to maintain physiological levels (about 15- to 20-fold higher serum levels) (Dohler *et al.*, 1979; Peer Review Panel, 1987).

D. Physiologic Actions of Thyroid Hormones

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

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

E. Regulation of Thyroid Hormone Synthesis/Secretion

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

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

The rate of release of TSH from the pituitary is delicately controlled by the amount of thyrotropin-releasing hormone (TRH) secreted by the hypothalamus and by the circulating levels of T_4 and T_3 . If for any reason there is a decrease in circulating levels of thyroid hormones, TSH is secreted and thyroid function is increased; if exogenous thyroid hormone is administered, TRH secretion is suppressed and eventually the thyroid gland becomes inactive and regresses. It appears that the plasma concentrations of both T_4 and T_3 (and possibly intracellular formation of T_3 from T_4 in the pituitary) are important factors in the release of TSH; they may also modulate the interaction of TRH with its re-

ceptors in the pituitary (Goodman and van Middlesworth, 1980; Hinkle and Goh, 1982; Larsen, 1982a; Ross *et al.*, 1986). Lastly, in the pituitary T_4 undergoes 5'-mono-deiodination to T_3 . In the rat about 50% of T_3 within pituitary cells arises from this means. When serum T_4 is reduced but T_3 is normal, pituitary intracellular T_3 is reduced and cells are able to respond to the decreased serum T_4 and increase TSH secretion (Larsen, 1982a).

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

Studies on the regulation of TSH output from the pituitary have indicated a link between T_3 nuclear receptor occupancy and the mRNA levels for the TSH subunit chains. Administration of exogenous T_3 resulted in decreases in TSH mRNA levels in the pituitaries and in transplanted pituitary tumors of thyroidectomized mice within 1 day of administration (Chin *et al.*, 1985). Subunit messenger RNA elongation in nuclei isolated from pituitary tumors of mice treated *in vivo* with T_3 decreased within 0.5 hr after hormone administration, and mRNA levels were reduced within 1 hr (Shupnik *et al.*, 1985). It appears that the decrease in mRNA is due either to decreased transcription or to decreased stability of the mRNA transcripts. A straight-line relationship existed between the proportion of nuclear T_3 receptors occupied and the proportional reduction in TSH subunit transcripts in transplanted pituitary tumors (Shupnik *et al.*, 1986). A 50% reduc-

tion in mRNA transcripts occurred when about 45% of the receptors were occupied; this occurred at plasma T₃ levels of about 1 ng/ml (1.5×10^{-9} M).

Other studies have investigated the effects of withdrawal of T₃ on TSH mRNA levels in thyroidectomized mice bearing transplanted pituitary tumors (Ross *et al.*, 1986). Plasma T₃ levels dropped precipitously within 1 day after withdrawal; plasma TSH concentrations rose fourfold between 1 and 2 days; and tumor TSH subunit mRNA levels increased markedly between Days 1 and 2.

These experiments demonstrate the rapid response of the pituitary gland to increases and decreases in plasma T₃ levels. It seems that pituitary cells modulate the levels in TSH subunit mRNAs as a function of the proportional occupancy of the numerous nuclear receptors for T₃.

II. THYROID AND PITUITARY GLAND NEOPLASIA

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

A. Thyroid Neoplasia

While, statistically, clinical thyroid cancer is not a serious human health problem in the United States, occult thyroid cancer discovered at autopsy (Sampson *et al.*, 1974) is much more common (average about 2% of autopsies). The American Cancer Society estimates there will be 11,000 new cases of thyroid cancer in 1988, which represents about 1% of the total expected cancer cases. In the same period it is expected there will be 1100

deaths from thyroid cancer, which is only 0.2% of the projected cancer deaths (Silverberg and Lubera, 1988). Overall, thyroid cancer 5-year relative survival rates are in excess of 90%. Although the trends in the average annual percentage change for thyroid cancer incidence has been increasing over the last 13 years (0.3%/year), the trend is not statistically significant (NCI, 1988). Other thyroid lesions, like "nodules" noted upon palpation of the thyroid, occur in about 4 to 7% of adults and are of concern to physicians because they may be or develop into thyroid malignancies (Paynter *et al.*, 1986; De Groot, 1979; Sampson *et al.*, 1974; Rojeski and Gharib, 1985).

1. Induction

Thyroid neoplasia may be induced by exposure of experimental animals to a variety of treatment regimens, exogenous chemicals, or physical agents. Some of these are discussed in more detail later. It has been recognized for some time that neoplasms induced in experimental animals by a number of these treatments result from thyroid gland dysfunction, in particular, hypothyroidism.

Among the thyroid cancer-causing factors inducing a hypothyroid state are iodine deficiency (Bielschowsky, 1953; Axelrod and Leblond, 1955; Schaller and Stevenson, 1966) and subtotal thyroidectomy (Dent *et al.*, 1956). In addition, thyroid tumors can result from the transplantation of TSH-secreting pituitary tumors (Dent *et al.*, 1956; Haran-Guera *et al.*, 1960; Sinha *et al.*, 1965). The one factor common to each of these conditions is that they all lead to increased production of TSH and prolonged stimulation of the thyroid gland by "excess" TSH. In the first two conditions, elevated TSH results from chronic stimulation of the pituitary in response to a deficiency in the circulating levels of thyroid hormone. Also note that nothing has been given to these animals: instead the tumors developed in the absence of something that is normally present (*i.e.*, iodine and thyroid gland mass). It should rightfully be

pointed out, however, that the animals are under chronic stress due to deficiency of thyroid hormone. In the third case, excess TSH comes from the transplanted pituitary tumor. Thus, irrespective of the cause, it appears that prolonged stimulation of the thyroid-pituitary feedback mechanism that results in release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia.

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

Still further support for the role of TSH in thyroid carcinogenesis comes from experiments using chemicals which reduce circulating thyroid hormone levels and result in increases in TSH (see Section III.B). Thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone that reestablish thyroid-pituitary homeostasis or by hypophysectomy (for examples see Yamada and Lewis, 1968; Jemec, 1980).

In general, thyroid neoplasms that have been induced in animals by excessive TSH stimulation remain dependent upon ongoing TSH stimulation, as when tissue fragments are transplanted from the original animals to a second host (see Doniach, 1970; for exception, note Ohshima and Ward, 1986). This is in keeping with the observation that thyroid

tumors in animals and humans retain their ability to respond to TSH in regard to differentiated cell functions and growth (Bielschowsky, 1955; Larsen, 1982b).

2. Morphological Stages in Thyroid Neoplasia

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

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

With continued TSH stimulation, the latent period is followed by a rapid and prolonged increase in thyroid weight and size. Although all thyroid tissue components proliferate to some extent, the major changes observed are associated with follicular cell hyperplasia. Thus, there are dramatic increases in both mitotic activity and in the number of follicular cells per gland (Wynford-Thomas *et al.*, 1982a). There are, however, limits to the extent to which thyroid hyperplasia, as well as thyroid weight and size, can continue to

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

Certainly, under experimental conditions of prolonged stimulation by TSH, diffuse thyroid hyperplasia may progress to a nodular proliferation of the follicular cells and eventually to neoplasia (Gorbman, 1947; Money and Rawson, 1950; Griesbach *et al.*, 1945; Doniach and Williams, 1962). While many of the resulting tumors are benign, prolonged and excessive thyroid stimulation may result

in malignant tumors. The morphology of thyroid tumors in laboratory rodents has been discussed in several reviews (Doniach, 1970b; Boorman, 1983; Frith and Heath, 1983). Studies with humans show a similar morphologic progression of the thyroid up through nodular hyperplasia and "adenomatous" lesions following prolonged stimulation by TSH (Ingbar and Woeber, 1981; see Section IV of this paper).

3. Reversibility of Morphological Progression to Thyroid Cancer

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

There is ample experimental evidence, however, showing that, to a significant though unknown extent, the morphological progression toward thyroid malignancy can be halted and at least partially reversed by removing the source of, and/or correcting for, the excessive thyrotropic stimulation. This may be achieved by administering adequate amounts of thyroid hormones to hypothyroid animals (Purves, 1943; Bielschowsky, 1955; Furth, 1969; Paynter *et al.*, 1986) or by effecting surgical hypophysectomy (Astwood *et al.*, 1943; MacKenzie and MacKenzie, 1943; Nadler *et al.*, 1970). Goiters in persons living in iodine-deficient areas tend to reverse following introduction of iodine in persons with hyperplasias of short duration (Ingbar and Woeber, 1981; see Section IV of this paper). In each case, these procedures counter the effect of the source of TSH stimulation.

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

In contrast, little or no indication of morphological reversibility was observed when rats that had received up to 500 ppm ethylene thiourea in their diets for a period of 2 years were returned to a control diet (Graham *et al.*, 1973). In another study (Bielschowsky and Goodall, 1963), methylthiouracil-induced thyroid lesions in the mouse continued to progress after goitrogen administration was stopped and replaced by thyroid hormone treatment. Most other studies indicate varying degrees of reversibility following discontinuation of goitrogen administration (Arnold *et al.*, 1983; Wollman and Breitman, 1970; Wynford-Thomas *et al.*, 1982c) or return of animals from a low iodine to a high iodine diet (Greer *et al.*, 1967).

In humans it has been common clinical practice to use high doses of thyroid hormone to try to suppress the growth of thyroid "nod-

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

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

B. Pituitary Neoplasia

Following chronic iodine deficiency (Axelrod and Leblond, 1955), treatment with goitrogens (Griesbach, 1941; Griesbach *et al.*, 1945), or surgical or ¹³¹I-induced thyroidectomy (Doniach and Williams, 1962; Carlton

and Gries, 1983), the anterior pituitary frequently exhibits a loss of acidophilic cells and an increase in basophil cells, and develops swollen "thyroidectomy cells," some of which contain cytoplasmic granules. These cells contain TSH (Osamura and Takayama, 1983) and, according to some researchers, may progress to TSH-secreting adenomas (Furth *et al.*, 1973; Bielschowsky, 1955), although other authors have failed to demonstrate tumors in such treated animals (for instance, see Ohshima and Ward, 1984, 1986). Pituitary hyperplasia and neoplasia appear to result from the same treatments causing thyroid neoplasia—conditions leading to prolonged circulating thyroid hormone decrease and excessive secretion of TSH by the pituitary gland.

C. Molecular Considerations in Thyroid Carcinogenesis

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

It is generally recognized that, under normal conditions, the control of cell division requires the interaction of a number of endogenous factors which work through a number

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

This section reviews available molecular information about the control of cell growth in thyroid cells and their conversion to neoplastic cells and attempts to incorporate this information into a plausible mechanistic framework. Although there are gaps in the understanding of the processes involved, what is known about the thyroid is consistent with the existing understanding of the components involved with the control of mammalian cell division. It is also consistent with current thinking that carcinogenesis is a multistep process and that multiple factors may influence its course. And finally, it accords special weight to TSH as playing a significant role in cell proliferation and in carcinogenesis of the thyroid gland.

1. Stimulation of Cell Division

a. Influence of TSH. TSH interaction with its receptor on the surface of the thyroid cell results in activation of adenyl cyclase and resultant production of cAMP, the activation of the phosphatidylinositol pathway, commencement of certain thyroid-specific differentiated functions that result in the formation of thyroid hormones, and stimulation of cell division. Although all cultured cells do not respond to TSH alone by increasing cell division (murine and canine do; porcine, ovine, and human do not [see Saji *et al.*, 1987]), the following steps have been identified in those that do respond. Almost imme-

diately (within 15 to 30 min) after addition of TSH to quiescent thyroid cells in culture, there are marked increases in the levels of the mRNAs for the cellular protooncogene, *c-fos*. A similar pattern is found for transcripts of the protooncogene, *c-myc*, but the induction is delayed somewhat, with the peak occurring at about 1 to 2 hr after TSH addition. These effects of TSH can be mimicked by direct addition of cAMP analogs or other factors that increase cellular cAMP (Dere *et al.*, 1985; Tammontano *et al.*, 1986a; Colletta *et al.*, 1986). Interestingly, human thyroid adenomas and carcinomas are characterized by *c-myc* expression, which is not found in the surrounding normal thyroid tissue. In addition, like normal cells in culture, adenoma cells respond to TSH in a dose-related manner by increasing the levels of *c-myc* transcripts (Yamashita *et al.*, 1986). This finding in human cells is in contrast to that cited above (Saji *et al.*, 1987).

The protein products of the *c-fos* and *c-myc* protooncogenes are thought to play a role in the replication of cells. Both *c-myc* and *c-fos* code for proteins that are largely restricted to the cell nucleus and appear to be functionally linked to DNA synthesis. The latter is illustrated by experiments showing that when monoclonal antibody to human *c-myc* protein is added to isolated nuclei, there is an inhibition of DNA synthesis and replicative DNA polymerase activity; the inhibition can be overcome by the addition of excess *c-myc* protein (Studzinski *et al.*, 1986). Further investigation is required in this area, since there are some questions about the original report (Gutierrez *et al.*, 1988; Studzinski, 1988).

There is additional evidence to indicate that oncogene expression may be an important factor in triggering cell division. For instance, certain human cancers have been shown to have chromosome rearrangements involving *c-myc*. This relationship has been well established for cases of Burkitt lymphoma (B-cell cancer) (Taub *et al.*, 1982; ar-Rushdi *et al.*, 1983; Nishikura *et al.*, 1983) and to a lesser extent for certain T-cell leuke-

mias (Erikson *et al.*, 1986; Finger *et al.*, 1986). It is thought that chromosomal translocations move *c-myc* to the regulatory units of immune response genes in these cells and bring about constitutive activation of the oncogene which then provides a continued stimulus for cell proliferation (see review by Croce, 1986), although recent evidence indicates that a number of Burkitt's cases also have point mutations at the binding site for a nuclear protein (Zajac-Kaye *et al.*, 1988).

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

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

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

Epidermal growth factor (EGF) is a naturally occurring polypeptide present in a number of organs that binds to specific receptors on sensitive cells. This binding results in activation of receptor-associated tyrosine kinase which phosphorylates the EGF receptor and

TABLE I
EFFECTS OF STIMULI ON THYROID CELLS

Stimulus	Enzyme activity	Induces <i>c-fos</i> and <i>c-myc</i>	Stimulates cell division	Effect on differentiated functions	Other
TSH	Adenyl cyclase	+	+	Enhances	Enhances EGF binding to its receptor
EGF	Tyrosine kinase	?	+	Inhibits	
TPA*	Protein kinase c	?	+	Inhibits	Inhibits EGF binding to its receptor and tyrosine kinase activity

* 12-*O*-tetradecanoylphorbol 13-acetate, a phorbol ester.

other sites and helps to bring about its cellular action. EGF is present in adult tissues; a related growth factor, transforming growth factor type α , is present in neoplasms and embryonic tissues and may be an embryonic form of EGF. It is interesting to note that one of the viral oncogenes, *v-erbB*, is a mutation of the EGF receptor gene where the binding-site portion of the receptor has been deleted, and that this mutation may result in constitutive activation, resulting in continued cell proliferation (Goustin *et al.*, 1986).

There is some work that indicates that EGF plays a role in the regulation of cellular activity and cell division in thyroid cells in culture. Its role *in vivo* needs to be ascertained. Unlike TSH, EGF blocks certain differentiated functions that typify thyroid action, such as formation of thyroglobulin by thyroid cells in culture (Westermarck *et al.*, 1983; Bachrach *et al.*, 1985; Roger *et al.*, 1986). In *in vivo* studies, infusion of sheep over a 24-hr period with EGF resulted in a profound drop in serum T₄ and T₃ which started within 10 hr after commencing administration. Part of this reduction in circulating thyroid hormones appears to be due to their enhanced metabolism (Corcoran *et al.*, 1986). These authors cite other work which shows that thyroid hormone administration results in increased tissue levels and urinary excretion of EGF. It thus seems that some feedback exists between levels of EGF and thyroid hormones.

EGF also produces increases in cell division in thyroid cells. By about 1 day after addition of EGF to thyroid cells in culture, there is stimulation in DNA synthesis (Westermarck *et al.*, 1983; Roger *et al.*, 1986), as was seen after administration of TSH. TSH increases the binding of EGF to its receptor on thyroid cells and, in combination with EGF, enhances DNA synthesis above that seen with EGF alone (Westermarck *et al.*, 1986).

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

Phorbol esters, like EGF, inhibit differentiated thyroid cell functions and stimulate cell division. As in other cells (Friedman *et*

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

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

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

Although studies on thyroid cells indicate that TSH, EGF, phorbol esters, and somatomedin C (and insulin) can each stimulate cell division in cultured thyroid cells, it does not mean that these factors are the only ones. For instance, many of the culture systems used in these studies included serum, which is known to contain a number of growth factors. In other cases, the culture medium was supplemented with hormones, growth fac-

tors, and other substances (e.g., somatostatin, cortisol, transferrin) which are known to effect cell cycle traverse (Bachrach *et al.*, 1985; Colletta *et al.*, 1986; Westermarck *et al.*, 1983).

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

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

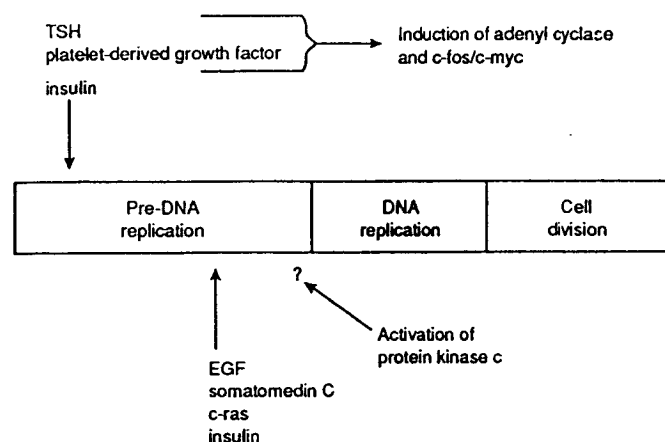


FIG. 4. Possible control points for cell division in the pre-DNA synthetic portion of the cell cycle.

Insulin (and related substances) seems to play a facilitating role in the thyroid. Alone in high concentrations it can induce thyroid cells in medium without serum to synthesize DNA, and it enables TSH to enhance this effect (Wynford-Thomas *et al.*, 1986). Insulin is active at both control points in certain mouse 3T3 cells as well (Rozengurt, 1986).

A model can be constructed for control of cell division in the thyroid gland (Fig. 4) that includes the two pre-DNA synthetic steps. The model engenders the known effects of various factors on thyroid cells and reflects certain observations in other mammalian cell systems. Although the model is not fully satisfactory, due to the inconsistencies across cell systems, it depicts certain interactions that may exist in the thyroid gland and suggests possible future research directions.

2. Cellular Transformation

As with the control of cell division, complex interactions among different factors seem to be operating during the transformation of normal to altered cells with neoplastic potential. Although activation of a single oncogene may not be sufficient in all cases to

produce transformation, activation of two different oncogenes is commonly sufficient to transform cells (see reviews by Weinberg, 1985; Barbacid, 1986). Frequently the cooperation includes the coordinate expression of an oncogene whose product is localized to the nucleus (e.g., *c-fos*, *c-myc*) with one whose product is in the cytoplasm (e.g., *c-ras*, *c-src*). As was mentioned previously, nuclear oncogenes can be activated by chromosomal translocation of the oncogene to cellular regulatory sequences; other activation mechanisms include the insertion of viral regulatory segments next to the nuclear oncogene, gene amplification (increase in the number of copies of the oncogene per cell), and stabilization of the oncogene gene product. On the other hand, cytoplasmic oncogenes tend to be activated by point or chromosomal mutations which affect the structure of their gene products (Weinberg, 1985).

TSH enhances *c-fos* and *c-myc* expression that may in turn interact with other factors in bringing about cell transformation. If the stimulus for TSH secretion from the pituitary is long term, as in the case of continued exposure to an antithyroid substance, it seems possible there could be continued oncogene transcription and a continued emphasis on

cell proliferation which could result in hyperplasia. Still other stimuli (e.g., activation of a second oncogene, certain point or structural mutations, interplay with growth factors) may aid in the transformation process and bring about neoplasia.

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

Studies on the thyroid gland are consistent with the idea that *c-myc* (through TSH stimulation) may interact with other stimuli in bringing about cell transformation. For instance, an enhancement of the carcinogenic response is noted when a treatment that increases TSH (e.g., iodide deficiency) follows application of a genotoxic agent (e.g., irradiation, nitrosamine) (see Section IV.B.4) which might produce a mutation that activates a second oncogene or some other effect.

One is still faced, however, with the observation that treatments that ensure prolonged TSH stimulation, as have been discussed previously, lead to neoplasia. Three possibilities exist: (1) TSH simply enhances spontane-

ously occurring events (e.g., mutations in regulatory sequences like oncogenes). The finding of thyroid neoplasms in about 1% of some untreated laboratory animals (Hasegan *et al.*, 1984) is in keeping with the idea that "spontaneous mutations" might exist in control animals that might predispose animals for development of thyroid tumors. (2) Through its effect on cell division, TSH may expand the thyroid cell population at risk for a spontaneous event and then promote neoplasia once a spontaneous mutation occurs. (3) TSH alone, via some yet undisclosed mechanism, might produce cellular transformation.

III. EXOGENOUS FACTORS INFLUENCING THYROID-PITUITARY CARCINOGENESIS

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

A. Physical Factors

External ionizing radiation is a known thyroid carcinogen in humans and experimental animals (NAS, 1980). Internal radiation, following administration of ^{131}I (a β - and γ -radiation emitter), produces thyroid tumors

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

B. Chemical Factors

1. Goitrogens

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

a. Naturally occurring (dietary) substances. These materials have been reviewed in detail by Van Etten (1969). The early observations of goiters in rabbits maintained on cabbage leaf diets (Chesney *et al.*, 1928) were followed by the discovery that the seeds of rape and other brassica species (cabbage, brussels sprouts, turnips, and mustard) also contained substance(s) that were goitrogenic when incorporated into rat diets (Hercus and Purves, 1936; Kennedy and Purves, 1941). Prolonged

dietary exposure to rape seed led to the development of adenomatous goiters (100% in 27 months) in rats (Griesbach *et al.*, 1945). L-5-Vinyl-2-thioxazolidone (goitrin) has been identified as the active goitrogen in turnips and the seed and green parts of other cruciferous plants. Goitrin from these sources may be passed to humans in the milk of cows feeding on such plants. In humans, goitrin appears to be about as active as propylthiouracil (Haynes and Murad, 1985). Peanuts are also reported to be goitrogenic in rats (Srinivasan *et al.*, 1957), the active component being the glucoside, arachidoside.

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

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

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

amides, to increase thyroid neoplasms in rats (Weisburger *et al.*, 1984).

(iii) Polyhydric phenols: The antithyroid activity (hypothyroidism and goiter) of resorcinol was first observed following the use of this material for treatment of leg ulcers in humans (Haynes and Murad, 1985). Subsequent studies have established that antithyroid activity is associated with compounds with *meta* polar-substituents on the benzene ring. Thus, hexyresorcinol, phloroglucinol, 2,4-dihydroxybenzoic acid, and *meta*-aminophenol are active, whereas catechol, hydroquinone, and pyrogallol are not (Paynter *et al.*, 1986).

c. Modes of action. Antithyroid agents belonging to structural groups (i), (ii), or (iii) all exert at least part of their activity by direct interference with the synthesis of thyroid hormone in the thyroid gland. All appear to block the incorporation of iodine into tyrosyl residues of thyroglobulin and by inhibiting the coupling of the idotyrosyl residues into idothyronines. It was proposed by Taurog (1976) that the antithyroid agents inhibit the enzyme peroxidase that is responsible for the conversion of iodide to the iodinating species and the subsequent iodination and coupling of the tyrosyl residues. This has been confirmed by subsequent studies (Davidson *et al.*, 1978; Engler *et al.*, 1982) showing that the compounds bind to and inactivate peroxidase when the heme of the enzyme is in the oxidized state. It is likely that these compounds show some inhibitory selectivity toward the different peroxidase-catalyzed reactions (i.e., iodination vs coupling) (Haynes and Murad, 1985). There is also evidence that some of the compounds (e.g., propylthiouracil) inhibit the peripheral deiodination of T₄ and T₃ (Geffner *et al.*, 1975; Saberi *et al.*, 1975).

Because of their ability to inhibit thyroid hormone synthesis, all of the above compounds have the potential to reduce circulating levels of T₄ and T₃ and, consequently, to induce the secretion of TSH by the pituitary. As a result, prolonged exposure to such compounds can be expected to induce thyroid

gland hypertrophy and hyperplasia and ultimately may lead to neoplasia.

2. Enzyme Inducers

In addition to chemicals exerting effects directly at the thyroid, as was summarized in the previous section, a number of others acting at peripheral sites can cause equally profound disturbances in thyroid function and morphology. Of particular interest are those compounds that induce hepatic and/or extrahepatic enzymes responsible for the metabolism of many endogenous and exogenous compounds. These chemicals can increase the metabolism of thyroid hormone, can result in a reduction in circulating thyroid hormone, and can stimulate an increase in TSH. Following long-term exposure to these agents, the thyroid gland undergoes hypertrophy and hyperplasia and finally, neoplasia.

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

(ii) Induction: Enzyme induction refers to the phenomenon whereby exposure of an animal to a given foreign compound results in the enhanced activity through *de novo* synthesis of a spectrum of the enzymes involved

in Phase I and Phase II metabolism (Cooney, 1967). Induction typically results in an increase in the rate at which the inducer and other compounds are metabolized and excreted.

Since the enzymes responsible for foreign-compound metabolism are thought by many to have evolved as a biochemical defense against potentially harmful environmental chemicals (Wilkinson, 1984), induction may be viewed as a biological adaptation that can provide important short-term benefits for survival. On the other hand, in light of increasing evidence that the enzymes detoxifying one chemical may activate another (Cummings and Prough, 1983), there has been concern that enzyme induction may represent a mechanism through which potentially dangerous toxicological interactions can occur following chemical exposure.

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

(iii) Different inducer types: Inducers of the enzymes involved in foreign-compound metabolism have been divided into at least two different categories on the basis of their characteristic effects on cytochrome P450 and monooxygenase activity (Mannering, 1971; Lu and West, 1978, 1980; Ryan *et al.*, 1978). One of these, typified by phenobarbital, led to a significant increase in liver size and weight and caused the substantial proliferation of hepatic endoplasmic reticulum. Induction was associated with increases in cytochrome P450 and a large number of monooxygenase reactions that enhanced metabolic (oxidative) capability toward many foreign compounds. The spectrum of oxidative reactions induced is now known to result mainly from the induction of one major isozyme of

cytochrome P450 that, in rats, is referred to as cytochrome P450b (Ryan *et al.*, 1978). A large number of drugs and other foreign compounds, including the chlorinated hydrocarbon insecticides (DDT and its analogs and the cyclodienes like chlordane and aldrin), exhibit induction characteristics similar to phenobarbital and are generally referred to as "PB-type" inducers.

Early studies with the polycyclic hydrocarbon, 3-methyl cholanthrene (3MC), clearly indicated that the induction characteristics of this compound were quite distinct from those of PB (Mannering, 1971). In contrast to the latter, treatment of animals with 3MC did not cause large increases in liver size or in the proliferation of endoplasmic reticulum; neither did it result in large increases in cytochrome P450. Instead, 3MC resulted in the formation of a qualitatively different form of cytochrome P450, known generally as cytochrome P448 and now referred to in rats as cytochrome P450c (Mannering, 1971; Lu and West, 1978; Ryan *et al.*, 1978). This cytochrome is associated with a rather limited number of oxidative reactions, the best known of which is aryl hydrocarbon hydroxylase (AHH) (Ryan *et al.*, 1978; Eisen *et al.*, 1983; Conney, 1982). AHH has received a lot of attention in recent years because of its role in the metabolic activation of compounds like benzo[*a*]pyrene to potent carcinogens (Eisen *et al.*, 1983; Conney, 1982). Inducers of the "3MC-type" include a number of polycyclic aromatic hydrocarbons, naphthoflavone, and several halogenated dibenzo-*p*-dioxins. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most effective inducer of this type to be discovered (Poland and Glover, 1974). The mechanism of action of inducers of this type involves high affinity binding to a cytosolic receptor and subsequent migration of the inducer-receptor complex to the nucleus where the transcriptional effect leading to enhanced protein synthesis is initiated (Eisen *et al.*, 1983). Induction of this type is genetically controlled by the so-called Ah locus in rodents and, while the true identity of the cytosolic receptor remains unknown, it is

hypothesized to be a receptor for some hormone or other physiologically important ligand.

While the PB-type and 3MC-type inducers still constitute the two major categories of inducers, it is now recognized that a number of other types exists, each characterized by increased levels of a distinct spectrum of isozymes of cytochrome P450 and other enzymes. It is also apparent that a number of compounds share some of the characteristics of more than one group and cannot be strictly classified. Technical mixtures of polyhalogenated biphenyls (PCBs and PBBs), for example, exhibit characteristics of both PB- and 3MC-type inducers (Alvares *et al.*, 1973), probably due to the presence in the mixtures of a number of isomers representing each type.

In addition to inducing a characteristic spectrum of isozymic forms of cytochrome P450, many of the inducers also result in enhanced titers and activities of other enzymes involved in foreign-compound metabolism. While these have not been well documented, they include epoxide hydratases, glutathione (GSH)-*S*-transferases, and several of the transferases (UDP-transferases, sulfo-transferases) associated with secondary conjugation reactions (Jacobsen *et al.*, 1975; Lucier *et al.*, 1975; Ecobichon and Comeau, 1974). It has been suggested that, like cytochrome P450, these enzymes may also exist in multiple isozymic forms and that different inducers may enhance the activity of specific isozymes with a characteristic range of substrate specificities.

b. Metabolism of thyroid hormones. The liver not only constitutes a target tissue for the thyroid hormones, but is also an organ responsible for the metabolic inactivation of the hormones and their elimination from the body. About half the T_4 elimination from the body of the rat occurs via the bile, whereas in humans only about 10 to 15% is lost in this way (Oppenheimer, personal communication 1987). While there appear to be quantitative differences in the relative rates of elimination of T_4 and T_3 , it is probable that both

are excreted by a qualitatively similar mechanism. The major pathway of elimination involves conjugation of the phenolic hydroxyl group of T_4 with glucuronic acid and biliary excretion of the resulting glucuronide (Fig. 1) (Galton, 1968; Bastomsky, 1973); sulfate conjugates may also be produced and excreted. On entering the intestine a portion of the conjugate may undergo hydrolysis by intestinal bacteria to release free thyroid hormone that may be reabsorbed into the circulation; this process is referred to as enterohepatic circulation. Unhydrolyzed conjugate cannot be reabsorbed and is excreted in the feces (Houk, 1980).

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

The effects of PB-type inducers on thyroid function are now known to be quite complex

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

Several studies have been conducted on the effects of PB-type inducers on thyroid hormone status in healthy human volunteers or in patients on different drug regimens. Drugs studied include phenobarbital, carbamazepine, rifampicin, and phenytoin (diphenylhydantoin). Most of the studies report decreased serum levels of T_4 (both protein-bound and free) (Rootwelt *et al.*, 1978; Faber *et al.*, 1985; Ohnhaus and Studer, 1983), but

reports vary on the changes observed in serum levels of T_3 and rT_3 depending on the type and concentration of the inducer employed. Ohnhaus and Studer (1983) observed a relationship between increasing levels of microsomal enzyme induction and decreasing serum levels of T_4 and rT_3 in healthy volunteers treated with combinations of antipyrine and rifampicin. An effect was only observed, however, at induction levels that decreased the half-life of antipyrine by more than 60%. Induction of hepatic enzymes is apparently only one of several mechanisms through which diphenylhydantoin can reduce circulating levels of T_4 (Smith and Surks, 1984). Other possible mechanisms by which diphenylhydantoin might act include serum protein displacement of the thyroid hormones, effects on the binding and biological activity of T_3 , and even effects on hypothalamic and pituitary regulation of TSH. Despite significantly decreased serum levels of T_4 , there seem to be a few reports of humans being placed in a hypothyroid condition as a result of treatment with drugs that induce liver microsomal enzyme activity. An exception is the observation that persons being maintained on exogenously supplied thyroid hormone become hypothyroid when given diphenylhydantoin or phenobarbital unless their thyroid hormone doses are changed (Oppenheimer, personal communication 1987). Furthermore, TSH levels never change significantly from those observed in the controls.

(ii) 3MC-type inducers: The effects on the thyroid of 3MC-type hepatic enzyme inducers (polycyclic aromatic hydrocarbons, TCDD, etc.) are perhaps the best understood of the compounds under discussion. A major mechanism involved seems to be the induction of the T_4 -UDP-glucuronyl transferase that constitutes the rate-limiting step in the biliary excretion of T_4 (Bastomsky, 1973). The effect is particularly well illustrated with reference to a variety of thyroid hormone parameters 9 days after treatment of rats with a single dose of 25 $\mu\text{g}/\text{kg}$ TCDD (Bastomsky, 1977a). Biliary excretion of ^{125}I (during the

first hour after injection of $^{125}\text{I}-T_4$) and the biliary clearance rate of plasma $^{125}\text{I}-T_4$ were increased about 10-fold. Somewhat unexpectedly, the biliary excretion of T_3 was unaffected by TCDD. As a direct consequence of these changes in metabolism and excretion, serum T_4 concentrations (but not those of T_3) were reduced to half those in controls. Other workers have reported decreased serum T_4 concentrations following TCDD treatment of rats (Potter *et al.*, 1983; Pazdernik and Rozman, 1985; Rozman *et al.*, 1985). TCDD treatment elevated serum concentrations of TSH and, as a result, produced thyroid goiters (measured by elevated thyroid weight) and enhanced ^{131}I uptake by the thyroid. There are conflicting reports as to whether TCDD enhances bile flow (Bastomsky, 1977a; Hwang, 1973), but this does not seem to be a major factor in its goitrogenic action. Interestingly, in hamsters, a species resistant to the acute toxic effects of TCDD, administration of the chemical raised T_4 and T_3 levels (Henry and Gasiewicz, 1987).

While TCDD is an unusually potent inducer of UDP-glucuronyl transferases, it appears to be at least somewhat similar to compounds such as 3MC (Bastomsky and Papatrou, 1973; Newman *et al.*, 1971), 3,4-benzo[*a*]pyrene (Goldstein and Taurog, 1968), and the polychlorinated and polybrominated biphenyls (PCBs and PBBs) (see below), all of which have been shown to enhance the biliary excretion of T_4 at least partly by increasing the formation of T_4 -glucuronide. TCDD did not uniformly increase hepatic UDP-glucuronyl transferase activity toward all substrates; it enhanced activity toward *p*-nitrophenol about fivefold but not toward testosterone or estrone. At the single dose of TCDD which produces maximal induction of mixed function oxidase activity in the livers of rats and hamsters there is about a 25–30% increase in transferase activity toward T_4 (Henry and Gasiewicz, 1987).

Recently, some investigators have suggested that the explanation for the interactions of TCDD with thyroid hormone levels is that T_4 and TCDD have common molecu-

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

(iii) Mixed-type: Perhaps as a result of their widespread contamination of the environment and their well-documented occurrence in human foods, the toxicological properties of PCBs and PBBs have received considerable attention (Kimbrough, 1974).

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

of iodide by peroxidase. More work in this area is needed.

Instead of comprising a single layer of cuboidal or low columnar epithelium, the follicular cells of PCB-treated animals became more columnar with multiple layers and hyperplastic papillary extensions into the colloid. Similar follicular cell hyperplasia has been reported in other chronic (Norris *et al.*, 1975) and subchronic studies (Sleight *et al.*, 1978) with PBBs. The histological changes, which are similar to those observed in animals treated with TSH (Seljeld *et al.*, 1971), were accompanied by substantially decreased (> threefold) serum thyroxine levels in PCB-treated rats (Collins *et al.*, 1977). Residual effects were observed 12 weeks after termination of exposure, probably reflecting the persistent nature of the PCBs. However, it is important to note that, even in animals exposed to the highest doses of PCBs, both the histological and functional abnormalities were reversible and were minimal 35 weeks after cessation of treatment.

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

(a) Increased biliary excretion (about 5-fold) and bile:plasma ratio (about 12-fold) following injection of $^{125}\text{I-T}_4$.

(b) Increased biliary clearance rate of plasma $^{125}\text{I-T}_4$ (more than 20-fold).

(c) Modest increase in bile flow (less than 2-fold).

(d) Decreased total serum and free T_4 concentrations.

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

It is apparent from these data that PCBs have effects that are similar to both PB-type and 3MC-type inducers. PCBs are reported to be potent inducers of liver T_4 -UDP-glucuronyl transferase (Bastomsky and Murthy, 1976) and, as with the 3MC-type inducers such as TCDD, this undoubtedly accounts, at least partially, for the increased biliary excretion of T_4 . On the other hand, PCB also displaced the thyroid hormones from their binding proteins in the serum (Bastomsky, 1974; Bastomsky *et al.*, 1976), an effect usually associated more with PB-type compounds. Because of its PB-like activity, it is also possible that PCB enhances hepatic binding of T_4 . It may be a combination of the induction of T_4 -UDP-glucuronyl transferase and the displacement from serum-binding proteins that lead to such high bile:plasma ratios of T_4 following PCB treatment; much smaller T_4 bile:plasma ratios are observed with compounds like salicylate that effect displacement but not enzyme induction (Osorio and Myant, 1963). Conversely, the effects of changes in binding proteins on metabolism of thyroid hormone under steady-state conditions do not seem to have been studied, and at least some arguments can be mounted that would suggest that no change in metabolism would occur under those conditions.

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

The effects of PCB treatment on circulating levels of T_3 are clearly different from those of T_4 . It has been suggested that since T_3 is more active than T_4 and because it is generated peripherally by 5'-monodeiodination of T_4 , T_4 may be serving simply as a prohormone. It is now generally accepted, however, that T_4 does have intrinsic hormonal activity. It is of considerable interest to note that, in contrast to the case with T_4 , treatment of rats with PCB does not result in any marked change in total serum or free concentrations of T_3 . While this may result from a number of different factors (Bastomsky *et al.*, 1976), no completely satisfactory explanation has yet been proposed. There is some suggestion that the relatively constant circulating levels of T_3 might be due to enhanced thyroidal secretion and enhanced peripheral conversion of T_4 and T_3 in response to the PCB-induced hypothyroidism.

In summary, in addition to possible direct effects on the thyroid, mixed-type inducers such as the PCBs and PBBs have several effects that, either alone or in combination, reduce circulating levels of the thyroid hormones and cause the pituitary to release TSH. These are (a) an induction of T_4 -UDP-glucuronyl transferase, (b) a displacement of T_4 from serum proteins, and (c) an increase in bile flow.

3. Inhibitors of 5'-Monodeiodinase

Certain thionamides, in addition to their known inhibition of iodination and coupling of tyrosine moieties into thyroid hormone, have the ability to inhibit the peripheral conversion of T_4 to T_3 . This is due to effects on 5'-iodothyronine deiodinase, a monodeiodinase which specifically removes the 5'-iodine from substituted thyronines. The enzyme requires a sulfhydryl-containing cofactor for activity, and it appears that some of the thionamides interfere with the cofactor to affect enzyme activity (Larson, 1982b). Compounds like thiouracil, propylthiouracil, and methylthiouracil inhibit the monodeiodination of T_4 to

T_3 and as a result reduce urinary iodide excretion, raise serum T_4 levels, and reduce the hormone effectiveness of T_4 by reducing conversion to T_3 . Other thionamides, like thiourea and methimazole, and the thiocyanate ion do not result in reduced thyroid hormone effectiveness (Green, 1978).

The activity of 5'-monodeiodinase can also be reduced by competitive inhibition of the enzyme by certain iodinated compounds like the radiocontrast agents, iopanoic acid and sodium ipodate, and the antiarrhythmic, amiodarone (Borowski *et al.*, 1985; Larsen, 1982b). The color additive, FD&C, Red No. 3 (Peer Review Panel, 1987), may also fall into this category.

FD&C Red No. 3 has been shown to produce thyroid tumors in dosed rats. With inhibition of the 5'-monodeiodinase, treated animals under certain conditions showed elevated T_4 , lowered or normal T_3 , and elevated TSH serum levels. Also, since the 5'-monodeiodinase seems to metabolize rT_3 to a diiodo-derivative, inhibition of the enzyme by Red No. 3 leads to elevated rT_3 levels too (Larsen, 1982b; Peer Review Panel, 1987).

4. Direct-Acting Chemicals and Treatment Combinations

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

a. Direct-acting chemicals. A few compounds have been identified that produce thyroid tumors that are not known to influence thyroid-pituitary status (see Hiasa *et al.*,

1982), two of which are *N*-nitroso compounds. Rats given eight injections of *N*-methyl-*N*-nitrosourea (NMU) over a 4-week period developed thyroid tumors by Week 36 without any development of goiter (Tsuda *et al.*, 1983). Likewise, there was no evidence of diffuse follicular hyperplasia in rats given a single dose of NMU and observed at 33 weeks, even though some animals had thyroid neoplasms (Ohshima and Ward, 1984). In a similar way, *N*-bis(2-hydroxypropyl)nitrosamine (DHPN) administration for 8 weeks led to thyroid tumors by 20 weeks without any increase in thyroid weight (Hiasa *et al.*, 1982); this observation was confirmed in a second laboratory (Kitahori *et al.*, 1984). Both nitrosamines produce tumors at sites other than the thyroid.

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

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

Some have likened this response in the thyroid to the initiation-promotion (two-step) phenomena originally described for mouse skin. In that case, treatment with the first agent (initiator) confers a permanent change in cells, such that exposure (usually prolonged) to the second agent (promoter) results in neoplasms; reversal of treatments is

ineffective as to tumor production. Over time it has become generally recognized that carcinogenesis is a multistep process that usually includes an initiation step as well as a promotional phase (OSTP, 1985).

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

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

Direct thyroidal effect is not the only way chemicals produce reductions in circulating thyroid hormone. Enzyme inducers increase the removal of thyroid hormone from the

blood while inhibitors of 5'-monodeiodinase block the formation of T₃ from T₄; in turn, both of these result in stimulation of the pituitary gland to secrete more TSH. The result, again, of long-term exposure is hypertrophy, hyperplasia, and eventually neoplasia. Only a limited number of chemicals have produced thyroid follicular tumors in animals in the absence of some antithyroid effect.

C. Structure-Activity Relationships

1. Chemicals Producing Thyroid Neoplasms in Animals

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

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

TABLE 2

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

1. Thionamides
<i>N,N'</i> -Dicyclohexylthiourea
<i>N,N'</i> -Diethylthiourea
Trimethylthiourea
2. Aromatic amines
a. Single ring
3-Amino-4-ethoxyacetanilide
<i>o</i> -Anisidine hydrochloride
2,4-Diaminoanisole sulfate
HC Blue No. 1
b. Bridged double rings
4,4'-Methylenebis(<i>N,N</i> -dimethyl)benzenamine
4,4'-Methylenedianiline dihydrochloride
4,4'-Oxydianiline
4,4'-Thiodianiline
c. Miscellaneous
C.I. Basic Red 9 monochloride
1,5-Naphthalenediamine
3. Complex halogenated hydrocarbons
Aldrin
Chlordane
Chlorinated paraffins (C ₁₂ , 60% chlorine)
Decabromodiphenyl oxide
2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin
Tetrachlorodiphenylethane (<i>p,p'</i> -DDD)
Toxaphene
4. Organophosphorous Compounds
Azinphosmethyl

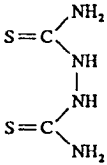
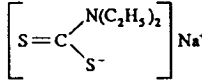
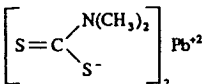
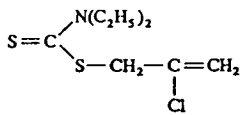
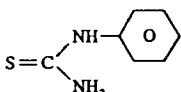
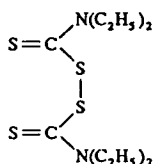
to effects on the thyroid. Thus, in 20 of 21 instances, there is some basis to think that thyroid neoplasms may be related to a reduction in thyroid hormone with a concomitant increase in pituitary stimulation of the thyroid through TSH.

Although most compounds producing thyroid neoplasms are members of specific chemical classes, not all members of those groups have been shown to produce such tumors. For instance, among the thionamides tested by NCI/NTP, *N,N'*-dicyclothiourea, *N,N'*-diethylthiourea, and trimethylthiourea yielded positive thyroid effects whereas several others did not (see Table 3).

It, therefore, seems reasonable to postulate that while a thionamide structure increases

TABLE 3

THIONAMIDES NEGATIVE FOR THYROID NEOPLASIA IN NCI/NTP STUDIES

1. 2,5-Dithiobiurea	4. Sodium diethylthiocarbamate
	
2. Lead dimethylthiocarbamate	5. Sulfallate
	
3. 1-Phenyl-2-thiourea	6. Tetraethylthiuram disulfide
	

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

2. Antithyroid Activity and Thyroid Carcinogenesis

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

Generally, the criteria for selecting the specific chemicals required that they had been (1) tested for animal carcinogenicity (NCI/NTP or IARC review) and (2) evaluated for antithyroid activity. However, in some cases

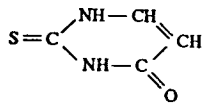
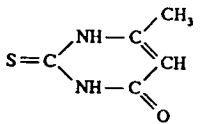
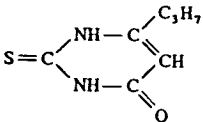
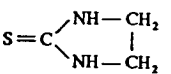
a chemical had been studied for carcinogenicity, but not antithyroid activity. In those cases, structurally related compounds that had been tested for antithyroid activity were chosen to act as surrogate indicators of a compound's antithyroid potential.

Antithyroid activity has been measured for a number of chemicals in rats and, to some extent, in humans. For rats, chemicals were administered orally at different doses for 10 days. Iodine concentrations in the thyroid were measured, and from the dose-response curve the dose that reduced the iodine concentration to a standard level was estimated (EDc). For comparison, the dose of thiouracil (a well-studied antithyroid agent) that reduced iodine concentration to the same level was also estimated (EDt). Antithyroid activity was expressed as the ratio of the estimated dose of thiouracil relative to that for the chemical (EDt/EDc), where thiouracil (in this review) is given a value of 100 (Astwood *et al.*, 1945; McGinty and Bywater, 1945a,b).

For humans, antithyroid activity for a chemical was again measured against the

TABLE 4A

THIONAMIDES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENICITY

Heterocyclic compounds	Relative antithyroid activity (thiouracil = 100)		Neoplasms ^d		
	Rat ^b	Human ^c	Thyroid ^e		Other sites
			Rat	Mouse	
1. 2-Thiouracil 	100	100	+	+	Mouse-liver
2. 6-Methylthiouracil 	100	100	+	+	Mouse-liver and pituitary
3. 6-n-Propylthiouracil 	1100	75	+	+	Mouse-pituitary
4. Ethylene thiourea 	40	50	+	+	Mouse-liver

^a From IARC reviews.

^b Astwood *et al.* (1945).

^c Stanley and Astwood (1947).

^d Mouse study did not examine thyroid.

^e McGinty and Bywater (1945a).

^f Not tested = n.

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

a. Thionamides. For the heterocyclic thionamides there is strong support for the premise that there may be a correlation between a chemical's ability to induce thyroid tumors and its ability to significantly inhibit iodine localization in the thyroid of rats and humans (Table 4A). For the thiourea-like thionamides (Table 4B), namely thiourea, trimethylthiourea, and *N,N*-diethylthiourea, relative antithyroid activities of about 10 or more were associated with thyroid tumor induction. In keeping with a correlation be-

TABLE 4B

THIONAMIDES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENICITY

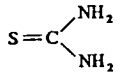
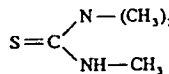
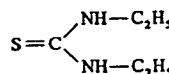
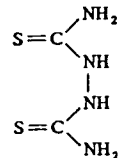
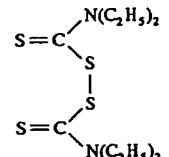
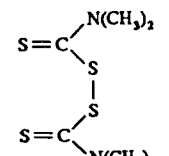
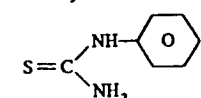
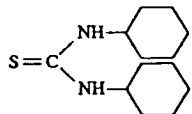
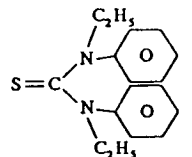
Thiourea derivatives	Relative antithyroid activity (thiouracil = 100)			Neoplasms ^d		
	Rat			Thyroid		
	ABH ^b	MB ^c	Human ^c	Rat	Mouse	Other sites
1. Thiourea 	12	9	100	+	+	Rat-liver, head, face Mouse-skull
2. Trimethylthiourea 	10	n ^f	n	+	-	-
3. <i>N,N'</i> -Diethylthiourea 	40	47	n	+	-	-
4. 2,5-Dithiobiurea 	1	n	n	-	-	-
5. Tetraethylthiuram disulfide 	n	n	n	-	-	-
6. Tetramethylthiuram disulfide 	1	n	n	n	n	n
7. 1-Phenyl-2-thiourea 	n	14	n	-	-	-

TABLE 4B—Continued

Thiourea derivatives	Relative antithyroid activity (thiouracil = 100)			Neoplasms ^d		
	Rat			Thyroid		
	ABH ^b	MB ^c	Human ^c	Rat	Mouse	Other sites
8. <i>N,N'</i> -Dicyclohexylthiourea 	n	n	n	±	-	-
9. 1,3-Diethyl-1,3-diphenylthiourea 	1	n	n	n	n	n

^a From NCI studies, except thiourea (IARC review).

^b Astwood *et al.* (1945).

^c Stanley and Astwood (1947).

^d Mouse study did not examine thyroid.

^e McGinty and Bywater (1945a).

^f Not tested = n.

tween these effects, 2,5-dithiobiurea and tetraethylthiuram disulfide (with its structural analog, tetramethylthiuram disulfide) both lacked antithyroid activity and did not produce thyroid neoplasia.

On the other hand, two other chemicals in the series of thiourea-like compounds need clarification. In the case of 1-phenyl-2-thiourea, a relative antithyroid value of 14 was found in rats, but the long-term NCI study in rats and mice was negative for thyroid tumors or thyroid hyperplasia. There was an absence of any toxic manifestations in dosed rats in the long-term study and a question whether a maximum tolerated dose had been used. In addition, after 78 weeks of chemical administration, dosed animals were observed for an additional 26 weeks in rats and 13 weeks in mice before termination. Since thyroid hyperplasia is often times reversible, it is possible that any lesions produced by dosing may

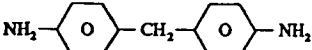
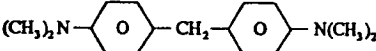
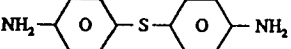
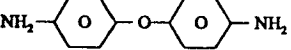
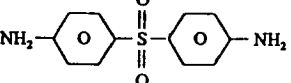
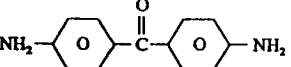
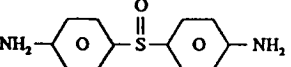
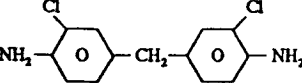
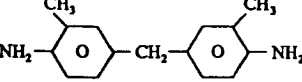
have regressed during the observation period. Other investigators have reported thyroid hyperplasia after 6 weeks of phenylthiourea administration to rats (Richter and Clisby, 1942), indicating that the chemical may induce thyroid neoplastic effects under certain conditions. Further work on this compound may bear this out.

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

b. Bridged double ring aromatic amines. Like the thionamides, certain aromatic amines with double rings attached by a simple ether-

TABLE 5

AROMATIC AMINES: RELATIONSHIP BETWEEN ANTITHYROID ACTIVITY AND THYROID CARCINOGENESIS

Bridged double ring compounds	Relative antithyroid activity: rat (thiouราซิล = 100)	Neoplasms ^a		
		Thyroid		Other sites
		Rat	Mouse	
1. 4,4'-Methylenedianiline dihydrochloride 	25 ^b	+	+	Mouse-liver Rat-liver
2. 4,4'-Methylenebis(N,N-dimethyl)benzenamine 	25 ^b	+	-	Mouse-liver
3. 4,4'-Thiodianiline 	15 ^c	+	+	Mouse-liver Rat-liver
4. 4,4'-Oxydianiline 	n ^d	+	+	Mouse-liver, hardenian gland Rat-liver
5. 4,4'-Sulfonyldianiline 	4 ^e	-	-	Rat-mesenchymal
6. Michler's ketone 	n	-	-	Mouse-liver
7. 4,4'-Diaminodiphenylsulfoxide 	12 ^f	n	n	n
8. 4,4'-Methylenebis(2-chloroaniline) ^g 	n	-	-	Mouse-liver, vascular Rat-liver, lung
9. 4,4'-Methylenebis(2-methylaniline) ^g 	n	-	n	Rat-liver

^a NCI/NTP bioassay except for last two chemicals in table.^b Astwood *et al.* (1945).^c McGinty and Bywater (1945b).^d Not tested.^e McGinty and Bywater (1946a).^f IARC review of carcinogenicity.

like bridge show a correlation between antithyroid activity and thyroid carcinogenesis (Table 5). 4,4'-Methylenedianiline, 4,4'-methylenebis(N,N'-dimethyl)benzenamine, and 4,4'-thiodianiline (chemicals No. 1 through 3, respectively) show both attributes, and although 4,4'-oxydianiline (No. 4) has not been tested for antithyroid activity, it has close structural similarity with the other three chemicals and also produces thyroid neoplasms. In keeping with its potential for antithyroid effects, chemical No. 4 produced increases in the number of TSH-secreting cells in the pituitary in rats following chronic administration (Murthy *et al.*, 1985), and chemicals No. 4 and No. 1 both produced thyroid enlargements in the NCI 90-day prechronic studies. All of these observations—antithyroid activity, thyroid enlargement in subchronic studies, and increases in the cell types of the pituitary that secrete TSH—are consistent with the hypothesis that bridged ring aromatic amines induce thyroid neoplasms by reducing circulating thyroid hormone levels and increasing TSH.

Other compounds in this series show results that are hard to interpret. 4,4'-Sulfonyldianiline (No. 5), which has an -SO₂- bridge between the rings, had a low antithyroid value of 4 in rats and was negative for thyroid tumors. Compound No. 6 with a -C(O)-bridge was also negative for thyroid tumors. Although chemical No. 7, which has an -S(O)-bridge, was negative for thyroid neoplasms, it was associated with an antithyroid value of 12 in the rat. Antithyroid values in the 10 to 15 range have been linked with positive thyroid tumorigenic effects for chemical No. 3 and some of the thionamides, e.g., thiourea. Further studies on antithyroid activity may help to clarify this inconsistency.

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

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

c. Characteristics of single ring aromatic amines. Many single ring aromatic amines have been evaluated for carcinogenicity in experimental systems and have shown positive effects (Clayton and Garner, 1976; Weisburger *et al.*, 1978; see review by Lavenhar and Maczka, 1985), but only a few of them have produced neoplasms in the thyroid. Of the single ring compounds that have been tested by NCI/NTP (Appendix B), *o*-anisidine (No. 1), 2,4-diaminoanisole (No. 2), 3-amino-4-ethoxyacetanilide (No. 3), and HC Blue No. 1 (No. 9) were the only ones to produce thyroid neoplasms. Of these agents only 2,4-diaminoanisole produced thyroid tumors in all four species-sex categories; the others produced such tumors in only one group.

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

3. Genotoxicity and Thyroid Carcinogenesis

It has been generally accepted by the scientific community that mutagenesis plays a role in carcinogenesis. In the case of thyroid follicular cell tumors, however, it has been suggested that a hormonal feedback mechanism

TABLE 6

GENOTOXICITY DATA FOR THIONAMIDES

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for thyroid tumors					
<i>N,N'</i> -Dicyclohexylthiourea	-	-	n	-	+
<i>N,N'</i> -Diethylthiourea	-	+	-	-	-
Trimethylthiourea	-	-	-	-	-
2. Chemicals negative for thyroid tumors					
1-Phenyl-2-thiourea	-	u	n	+	+
2,5-Dithiobiurea	-	n	n	-	+
Tetraethylthiuram disulfide	-	+	n	+	-
Sulfallate	+	n	n	n	n
Lead dimethyldithiocarbamate	+	u	-	+	+
Sodium diethyldithiocarbamate	-	+	n	-	-

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

involving increased output of thyroid-stimulating hormone from the pituitary gland in response to low thyroid hormone levels may be operating (Woo *et al.*, 1985; Paynter *et al.*, 1986). Even though hormone imbalance may play a role in thyroid carcinogenesis, it is also important to evaluate the mutagenic potential of agents causing these tumors.

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

This review largely draws upon those compounds that were tested in rats and mice for carcinogenicity by NCI/NTP and produced thyroid neoplasms. Structurally related compounds that did not produce thyroid tumors

are included for comparison. The genotoxicity data on these chemicals are from the NTP, much of which has not been published in peer-reviewed journals and at least some of which could be considered preliminary in nature.

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

TABLE 7

GENOTOXICITY DATA FOR SINGLE RING AROMATIC AMINES

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for thyroid tumors					
3-Amino-4-ethoxyacetanilide	+/+	n	-	n	n
<i>o</i> -Anisidine hydrochloride	+	n	n	n	n
2,4-Diaminoanisole sulfate	+/+	+/+	n	u	u
HC Blue No. 1	+	+	-	+	+
2. Chemicals negative for thyroid tumors					
<i>p</i> -Cresidine	+/+	n	n	n	n
5-Nitro- <i>o</i> -anisidine	+	n	?/-/?	n	n
<i>p</i> -Anisidine	-/+	n	n	w	+
2,4-Dimethoxyaniline hydrochloride	+	+	n	+	+
<i>m</i> -Phenylenediamine	+	n	n	+	+
<i>p</i> -Phenylenediamine hydrochloride	+	+/+	u	+	+
2-Nitro- <i>p</i> -phenylenediamine	+	+/+	n	+	+

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

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

The genotoxicity of ethylene thiourea, a compound known to produce thyroid tumors, was assessed in greater detail (see Appendix C). Although it was concluded from the journal articles that there is evidence for genotoxicity when ethylene thiourea is supplemented with sodium nitrite (*Salmonella* with metabolic activation, *in vivo* cytogenetics, dominant lethal, micronucleus), presumably via the formation of *N*-nitrosoethylene thiourea, there is much less evidence for the

genotoxic potential of ethylene thiourea itself. The compound shows little indication of gene mutation activity: negative to weakly positive effects in bacteria, negative in *Drosophila*, and conflicting information in yeast and mammalian cells in culture (negative in CHO cells and divergent results in mouse lymphoma cells). Chromosomal effects are not demonstrated in cells of higher eukaryotes in culture or *in vivo*. DNA damage tests showed conflicting results in bacteria, yeast, and human cells in culture.

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

TABLE 8

GENOTOXICITY DATA FOR BRIDGED DOUBLE RING AROMATIC AMINES

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for thyroid tumors					
4,4'-Methylenedianiline dihydrochloride	+	+	n	+	+
4,4'-Methylenebis(N,N-dimethyl)benzenamine	-	+/+	n	n	n
4,4'-Thiodianiline	+	n	n	u	u
4,4'-Oxydianiline	+	+	n	+	+
2. Chemicals negative for thyroid tumors					
Michler's ketone	+/+	+/+	n	-	-
4,4'-Sulfonyldianiline	-/-	-	n	+	+

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

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

b. Aromatic amines. Unlike thionamides, the class of aromatic amines commonly demonstrates genotoxic effects for both point mutations and chromosomal effects (Tables 7, 8, and 9). This is the case for chemicals that pro-

duced thyroid tumors as well as for analogs that did not.

The genotoxic potential of 4,4'-oxydianiline was evaluated in more detail using information from the published literature (Appendix D) to supplement that generated by NTP (Table 3). It is concluded that it is a frameshift and perhaps base-pair substitution mutagen in *Salmonella* that requires metabolic activation for an effect to be noted. In keeping with its mutagenic effects on bacteria, 4,4'-oxydianiline also produced gene mutations, chromosome aberrations, and sister chroma-

TABLE 9

GENOTOXICITY DATA FOR MISCELLANEOUS AROMATIC AMINES

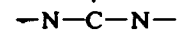
	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
Chemicals positive for thyroid tumors					
C.I. basic red 9 monochloride	+/?	+/?	n	-	+
1,4-Naphthalenediamine	+	n	n	n	n

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

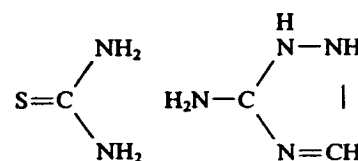
tid exchanges (SCE) in cultured mammalian cells. However, SCE are not increased *in vivo*, and two DNA damage assays *in vivo* gave discordant results. *In vitro* transformation studies were generally positive. Thus, the analysis of 4,4'-oxydianiline confirms the suspicion from Tables 7 through 9 that aromatic amines are genotoxic agents.

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

d. Amitrole. Amitrole has not been investigated by the NTP concerning its carcinogenicity, but from other long-term animal studies it is known to produce thyroid, pituitary, and liver tumors (see Paynter *et al.*, 1986). Like the thionamides and aromatic amines, amitrole inhibits thyroid peroxidase. Although it lacks the thiol group of thionamides, it does show some structural similarity (an R grouping), as illustrated with the



comparison with thiourea.



Thiourea Amitrole

Gene mutation testing of amitrole has spanned prokaryotes, yeast, insects, and

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

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

Tests for structural chromosome aberrations have been uniformly negative and include the following: human lymphocytes in culture, mouse bone marrow cytogenetics, and mouse micronucleus and dominant lethal tests.

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

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

Increases in unscheduled DNA synthesis have been reported in human cells. For HeLa cells, a positive dose-response effect for amitrole was noted in the presence of rat liver S9; no such increase was noted in the absence of

TABLE 10
GENOTOXICITY DATA FOR COMPLEX HALOGENATED HYDROCARBONS

	Gene mutations			Chromosomal effects	
	SA	ML	SLRL	CA	SCE
1. Chemicals positive for thyroid tumors					
Aldrin	s	n	n	n	n
Chlordane	(r)	(t)		(r)	(r)
	-	+	n	-	+
Chlorinated paraffins (C ₁₂ , 60% chlorine)	-	n	n	n	n
Decabromodiphenyl oxide	-	n	n	-	-
2,3,7,8-Tetrachlorodibenzo-p-dioxin	-	-	-	-	-
p,p'-Tetrachlorodiphenylethane (p,p'-DDD)	-	n	n	u	u
Toxaphene	+	n	n	n	n
2. Chemicals negative for thyroid tumors					
Dieldrin	-	+	n	-	+
Heptachlor	-	u	n	+	+
Chlorinated paraffins (C ₂₃ , 43% chlorine)	-	n	n	n	n
PBB mixture (Firemaster FF-1)	-	-	n	-	-
p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE)	-	+	+/-	-	w

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

exogenous activation (Martin and McDermid, 1981). Also, amitrole was reported in an abstract to be positive in human EUE cells; the conditions of the study were not given.

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

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

e. *Conclusion.* The review of three chemical classes demonstrating thyroid carcinogenesis il-

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

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

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

Although the remarks made in the previous paragraph are representative impressions of the data on chemical classes as a whole, they certainly do not necessarily apply to any one chemical within a class. Many times chemicals give a smattering of positive and negative results. In other cases, such as with the thionamides and amitrole, the evidence indicates a general lack of activity for some end points (e.g., gene mutations and chromosomal aberrations), but the potential presence for other effects (e.g., *in vitro* transformation). Each of these cases makes it difficult to reach an all-inclusive position on genotoxicity. Still, within the limits of the present review, there does not seem to be a consistent relationship across chemical classes that produce thyroid tumors as to their ability to produce genotoxic effects.

IV. HUMAN DATA ON THYROID HYPERPLASIA AND NEOPLASIA

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

different approaches and merging data from clinical observations, studies of clinical populations, and epidemiologic studies.

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

A. Thyroid-Pituitary Function

It is widely accepted that the pituitary-thyroid axis and the nature, body handling, and function of thyroid hormones and TSH are quite similar in experimental animals and humans. For instance, in a review of thyroid function in humans, Larsen (1982a) presented clinical data on the feedback regulation of thyrotropin secretion by thyroid hormones and the tissue conversion of T₄ to T₃ that is basically like that in experimental animals. Recent evidence, however, helps to point out some of the differences that may exist between animals and humans. For instance, in the rat there is active conversion of T₄ to T₃ which then regulates TSH production, whereas in humans circulating T₃ may play a more dominant role (Fish *et al.*, 1987).

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

Chemical	n	Dose or exposure ^a	Health status ^b	Effects ^{c,d}	Temporal ^e	Data base ^f	Ref.
Amiodarone	229 treated >83 cardiac	~270 mg/day >17 months	Chronic treatment for cardiac disorders, male and female; from Italy and Massachusetts. Cardiac and normal controls. 161 treated were euthyroid.	Italy: 10% hyperthyroidism, 5% hypothyroidism; Massachusetts: 2% hyperthyroidism, 22% hypothyroidism.	Response blunted with chronic treatment.	Other studies concur that hyperthyroidism is seen less in areas of sufficient I ⁻ intake. Relationship well characterized.	Martino <i>et al.</i> (1984)
Carbamazepine (CBZ)	27 83 controls	Dose not stated, CBZ alone or with phenobarbitone. Long term.	Adult epilepsy patients, long-term therapy. Controls euthyroid.	↓ T ₄ *; no change in T ₃ , no change in TSH.		One other report.	Rootwelt <i>et al.</i> (1978)
	7	CBZ alone	New patients, basal and treatment values.	↓ T ₃ and FT ₃ index*; ↓ FT ₄ index*; ↑ TSH* to Day 20 then ↓.	Perhaps delayed effect due to enzyme.		
Ethionamide	2	1 g/day plus other drugs	48-year-old female with TB; 54-year-old male with TB and diabetes.	↓ T ₄ , ↑ TSH, goiter in female	Symptoms and T ₄ normal after drug removal.	One other report had unclear etiology.	Moulding and Fraser (1970)
Ethylene thiourea	46 40 controls	In air 10-240 μg/m ³	Male workers and controls with no history of thyroid disease.	↓ T ₄ *; normal TSH. 1-hypothyroidism.	Lower T ₄ in more exposed group.	Three studies in workers, no or slight changes in thyroid function.	Smith (1984)
Lithium	86 105 controls	Male, 32 (18-48) mEq/day; female, 26.7 (8-48) mEq/day; 3-169 months.	Manic-depressive. Outpatient male and female.	↓ T ₃ ; ↓ T ₄ ; ↑ TSH in females; hypothyroidism.		Several studies report high frequency of hypothyroidism, especially in women; goiter reported only in females. TSH considered diagnosed.	Transbol <i>et al.</i> (1978)
Oxyphenbutazone	1	Not stated	63-year-old female with back pain.	↓ T ₃ ; ↑ I uptake; ↑ hypothyroidism.	Remission after drug removal.	First report; phenylbutazone known to be goitrogen.	Lane <i>et al.</i> (1977)
Phenytoin	10 83 controls	Not stated	Adult epilepsy patients on long-term treatment.	No change in T ₃ ; ↓ T ₄ *; no change in TSH.	Similar to carbamazepine therapy.	Literature agrees on ↓ T ₄ .	Rootwelt <i>et al.</i> (1978)
Polybrominated Biphenyls (PBB)	35 89 controls	Occupational exposure; >6 weeks, polybrominated biphenyl oxide	Males free of thyroid disease.	Low T ₄ ; ↑ TSH; no goiter; 4/35 hypothyroidism. Antithyroid antibodies in some.	↓ T ₄ may persist after exposure ceases.	No other reports. Thyroid abnormalities in rats given PBBs and polybrominated biphenyl oxide.	Bahn <i>et al.</i> (1980)
Sulphonyl-ureas	220	42-60 months treatment, average duration. 0.6-3.0 g/day tolbutamide or 0.1-0.5 g/day chlorpropamide	Diabetics with no blood urea. Groups age and sex matched.	↓ serum PBI, incidence with duration of treatment; 0 goiter; ↑ hypothyroidism.	PBI to normal after treatment stopped and drops again when treatment resumed.	Several other studies of ↓ PBI, ↓ in ¹³¹ I uptake; no hypothyroidism with short-term treatment. Carbutamide; more pronounced effects.	Hunton <i>et al.</i> (1965)
	229 controls	Diet alone, 113; insulin, 93; biguanides, 23.	Diabetics				
Resorcinol	3	Ointment on leg ulcers.	Females, 50, 59, 60 years. 1-cardiac, all clinical hypothyroidism cases.	↓ PBI in 2; rapid development of severe symptoms.	↑ ¹³¹ I uptake when treatment stopped and hypothyroidism reversed.	A 1977 report of hypothyroidism in a dialysis patient cites only this reference.	Bull and Fraser (1950)

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

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

^c Effects examined varied among studies. The column reports results of five items; serum T₃, T₄ and TSH; thyroid gland enlargement; clinical thyroid dysfunction. Other items examined are not reported in the table. Absence of entry indicates effect not assessed.

^d Symbols: *Statistically significant at <0.05. Specified only if test used is stated in text and appropriate. However, testing may vary among studies, e.g., most are tests of mean differences, but Bahn *et al.* (1980) test differences in number with elevated levels between groups. ↑, increase; ↓, decrease; T₃ and T₄, serum levels; hypo- and hyperthyroidism refer to clinical observation.

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

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

B. Causes of Thyroid Hyperplasia

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

1. Chemical Inhibitors

Several examples of chemical substances that influence thyroid status in humans are summarized in Table II to illustrate the nature of the effects. The agents include such things as thyroid peroxidase inhibitors (e.g., ethylene thiourea, sulphonylureas, resorcinol), a cation (lithium), an organiodide (amiodarone), and inducers of mixed function oxidases (phenobarbital, PBB). In each case exposures result in reduction in circulating thyroid hormone levels and in some cases elevated TSH levels or goiters. These responses are like those seen in animals.

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

The value of a case report in support of the hypothesis is strengthened if cessation of

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

Other observations point out the comparability of response in humans as in animals. In hypothyroid animals the cells of the pituitary enlarge and become "thyroidectomy cells" (Baker and Yu, 1971) and, according to some authors, may undergo hyperplasia and finally neoplasia (see Section II.B). Indirect studies in humans also demonstrate some of these findings. The bony covering of the human pituitary, the sella turcica, normally enlarges with age up to about 20 years and then remains essentially constant in size. Enlargement in the sella turcica beyond normal limits is noted in cases of hypothyroidism, and there is an inverse relationship between the blood levels of thyroid hormones and sella size and a direct one between TSH levels and size of the sella turcica (Yamada *et al.*, 1976; Bigos *et al.*, 1978). It is interesting to note that there are also a few clinical reports linking chemical hypothyroidism and pituitary adenomas, and at least some of them appear to be TSH-secreting tumors (e.g., Samaan *et al.*, 1977; Katz *et al.*, 1980; see review by Balsam and Oppenheimer, 1975), although the case is not established with any certainty.

2. Dietary Factors

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

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

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

Iodine deficiency in humans can result in profound thyroid hyperplasia. Goiters up to 5 kg (a 100-fold increase in weight) have been observed in iodine-deficient areas as a compensatory response to inability to synthesize thyroid hormone. Generally, the impairment in hormone synthesis is overcome in time and the individual becomes clinically euthyroid, even in the presence of some derangement in T_4 and TSH levels. Often in goitrous populations repeated cycles of hyperplasia and involution occur which can lead to multinodular goiter. In contrast to the hyperplastic goiter, multinodular goiters do not regress upon administration of iodine. Likewise, thyroid hormone usually has no effect on longstanding goiters (Ingbar and Woerber, 1981). Adenomatous hyperplasia is a less common cause of nodularity but is significant because

it is difficult to distinguish from neoplasia, thus complicating the assessment of the association between hyperplasia and neoplasia. As will be developed later in this section, it does not appear that thyroid cancer is a major problem arising from iodine-deficient goiters, in contrast to the observations in experimental animals which indicate that tumors frequently arise under iodine-deficient conditions.

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

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

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

It has been assumed, therefore, that eating foods producing the thiocyanate ion or goitrin contributes to endemic goiter. De Groot and Stanbury (1975) cite studies in Australia,

Finland, and England that suggest cattle have passed these goitrogens to humans through milk. Progoitrin has been detected in commercial milk in goitrous regions of Finland, but not in nongoitrous regions. Seasonal development of goiter in school children has been related to milk from cows fed kale (De Groot and Stanbury, 1975).

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

Recent studies in Africa contribute more direct evidence to support an interactive effect of TCN (or cyanogenic glucosides) and a diet low in iodine. In an iodine-deficient region of the Sudan where goiter prevalence may reach 55%, the frequency of large goiters is higher in rural than in urban areas (Eltom *et al.*, 1985). The predominant staple food in rural Darfur is millet. Rural subjects with goiters had statistically significantly higher levels of TSH and T₃ and lower levels of T₄ and free T₄ index than urban subjects with goiters. Serum TCN was significantly higher in rural subjects, but the elevated levels of urinary TCN did not reach statistical significance. The urinary iodine excretion, a reflection of quantity of iodine ingested, was not significantly different between the two groups. These results are consistent with the hypothesis that TCN overload in conjunction with iodine deficiency causes more severe thyroid dysfunction than iodine deficiency alone. Evidence of a possible effect has also been reported in North Zaire in Central Africa in children with iodine deficiency (Vanderpas *et al.*, 1984).

C. Causes of Thyroid Cancer in Humans

Epidemiologists search for clues to causes of disease and to factors that increase an individual's risk of disease (risk factors) by exam-

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

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

As a result of descriptive and analytic epidemiologic data, radiation is a well-documented cause of thyroid cancer in humans (Schottenfeld and Gershman, 1978; Ron and Modan, 1982). Incidence rates for thyroid cancer rose roughly twofold between the 1940s and the 1970s for persons under age 55. The change in pattern coincides with administration of X-ray for various medical treatments and is consistent with the hypothesis that ionizing radiation is a cause of thyroid cancer in children and young adults. Childhood irradiation was observed more often in thyroid cancer cases than controls. Ron and Modan (1982) summarize eight epidemiologic studies of populations exposed to X-ray therapy, atomic bomb explosions, and fallout from nuclear weapons testing.

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

perplasia and nodules than controls. These steps are summarized in the sections below.

1. Descriptive Epidemiology

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

For several countries, thyroid cancer shows rising age-adjusted incidence rates with age and consistently higher rates for women than men, particularly in young adults. Rates for males range from 0.6 to 5 per 100,000 and for females from 1.2 to 16 per 100,000. Variations by country are relatively small compared with that for other cancer sites (about 10-fold) and are not consistently related to geography or race. The highest age-adjusted rates in females (1967-1971) were for Hawaiians in Hawaii (16/100,000), Iceland (16.3/100,000), and Israeli Jews (8.3/100,000) (Waterhouse *et al.*, 1982).

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

Experimental evidence in several laboratory species demonstrates that iodine defi-

ciency, certain chemicals, and other causes of prolonged TSH stimulation result in thyroid enlargements and eventually thyroid tumors. In the absence of such information in humans other studies need to be conducted to get some handle on human thyroid carcinogenesis.

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

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

sexes were about three times higher in Colombia than in the other two sites.

Several reasons may account for differing study outcomes. Some of the correlations are based on reports of high thyroid cancer rates generated from pathology studies of surgery cases, and are likely to suffer from a selection bias because thyroid disease suspected of carcinogenicity is likely to be referred to surgery (De Groot and Stanbury, 1979). Different causes of cancer may result in different histopathological types of thyroid cancer. In the United States, in particular, radiation-induced cancer associated with therapy in childhood could have masked a decrease associated with iodine prophylaxis. After the introduction of iodized table salt in Switzerland and the decreasing incidence of goiter, thyroid cancer rates remained stable but an increasing proportion of thyroid cancers were classified as papillary (Shottenfeld and Gershan, 1977). Therefore, the conflicting data cited above are inconclusive and difficult to interpret.

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

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

Further evidence of a relationship between iodine intake (from inadequate to hypernormal) and the form of thyroid cancer comes

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

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

Probably the most profound disruptions in thyroid functioning occur in cases of familial goiter where there are inherited blocks in thyroid hormone production (Stanbury *et al.*, 1979). When left untreated, these patients develop profound hyperplasia and nodular (benign tumor) changes, but only a very few cases have gone on to develop thyroid carcinoma (see review by Vickery, 1981). Like with endemic goiter, it appears that the en-

TABLE 12

EPIDEMIOLOGIC STUDIES OF THYROID CANCER AND ITS RELATIONSHIP TO GOITER AND THYROID NODULES

Odds ratio (95% confidence limits) ^a		Comment	Ref.
Goiter	Thyroid nodules		
4.5 (1.6–12.2) ^b	8.7 (1.6–47.5) ^b	Women aged 18–80	McTiernan <i>et al.</i> (1984)
	10.5 (2.5–44.8) ^c	White women aged 15–40	Preston-Martin <i>et al.</i> (1987)
5.6 (1.0–41) ^d	33 (4.5–691) ^d	Adjusted for age, sex, and prior radiation exposure	Ron <i>et al.</i> (1987)

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

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

^c Presence of goiter or benign nodules.

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

larged thyroids in these patients do not often undergo malignant transformation; this contrasts with the findings in long-term animal studies where blocks in thyroid production regularly lead to thyroid cancer.

Although not much seems to have been done concerning the follow-up of patients with Graves' disease (hyperthyroidism) as to thyroid cancer development, the little that has been done (a follow-up of 30,000 patients) suggests there may not be a significant thyroid cancer problem in these cases (Dobyns *et al.*, 1974; see also Doniach, 1970a). [One very small study of Graves' patients suggested a higher than expected frequency of thyroid cancer (Shapiro *et al.*, 1970).] The reason Graves' patients may be at risk is the finding that many of the persons carry immunoglobulins in their blood which bind to the TSH receptor on thyroid cells and, at least *in vitro*, act like TSH to stimulate DNA synthesis and cell division (Valente *et al.*, 1983; Truitt *et al.*, 1986b). Since these patients frequently have enlarged thyroid glands, one cannot help but think that the immunoglobulins may stimulate thyroid cell division *in vivo* as well. A small number of cases of thyroid cancer in Graves' disease have recently been reported (Filetti *et al.*, 1988).

The single investigation of Graves' disease

patients treated with antithyroid agents (i.e., thionamides) for at least 1 year failed to show any thyroid cancers in over 1000 patients (Dobyns *et al.*, 1974). Again, this suggests that at least circumscribed use of antithyroid drugs is not attended with a marked thyroid cancer risk. It should be pointed out, however, that the goal of antithyroid treatment for Graves' disease is to bring patients into euthyroid and not a hypothyroid status where increases in TSH may occur. Thus, the follow-up of treated case of Graves' disease does not provide significant evidence to impugn or acquit antithyroid agents.

In the case of Hashimoto's thyroiditis, a common condition considered to be an autoimmune disorder, patients commonly have high circulating levels of TSH (Larsen, 1982b). The clinical impression is that the only association between this disease and thyroid cancer is with the thyroid lymphoma and not follicular cell carcinoma (Woolner, 1959).

2. Analytical Epidemiology

Of all the various types of data on humans from which causal associations can be inferred, the strongest evidence is derived from

analytical epidemiology—cohort or case-control studies—that evaluate data on individuals and suitable controls. Analytical epidemiologic studies have helped to establish ionizing radiation as a cause of thyroid cancer (Ron and Modan, 1982).

Three case-control studies of thyroid carcinoma in the United States have recently been completed which evaluated risk factors for cancer, including preexisting thyroid disease (Table 12). These studies were designed to test a potential hypothesized role of endogenous female hormones in thyroid cancer. Hormonal factors are suspected as a cause of thyroid cancer because of the consistently higher rates in females and the peak occurrence in females at between ages 15 and 29 when hormonal activity is enhanced (Henderson *et al.*, 1982; Ron and Modan, 1982). Each study showed significant increases in thyroid nodules and goiters among thyroid cancer patients.

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

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

There are some potential biases in the McTiernan *et al.* (1984) study such as recall bias, relatively low ascertainment rate (65%), the lack of reevaluation of the histopathology, and the reliance on telephone interviews

rather than medical history. However, it is doubtful that these could be the cause of associations of the magnitude noted.

Preston-Martin *et al.* (1987) conducted a case-control study in which they questioned 110 female cases aged 15 to 40 and an equal number of matched controls. Diagnoses of cases were histologically confirmed, and thyroid disease was recorded if a physician was consulted at least 2 years prior to the cancer diagnosis. Statistically significant risk factors were found for thyroid enlargement as an adolescent (OR = 10) and any goiter or benign nodules (OR = 10.5). The odds ratio of any thyroid disease was 14.5. The small number of cases of follicular carcinoma prevented analysis by histological type.

Ron *et al.* (1987) also found increased risk with parity as well as increased risk with goiter and nodules. This case-control study included 159 cases (109 female and 50 male) ascertained through a cancer registry and 318 controls from the general population. A review of the pathology was included. Thyroid nodules were evaluated separately from goiter and had a far greater risk (OR = 33) compared with goiter (OR = 5.6); both were statistically significant. The authors offer as caveats the fact that thyroid disease status was not medically verified and the response rate was only 62%.

In conclusion, these three recent case-control studies in the United States consistently showed thyroid cancer strongly related to preexisting goiter and to thyroid nodules (Table 12). There is insufficient evidence to identify a quantitative difference in this relationship between follicular or papillary tumor types. One concern is that the association between thyroid disease and thyroid cancer may be increased as a result of closer medical attention; after all, there must have been some clinical indication that the patients may have had a thyroid neoplasm prior to the time of surgery (like the presence of a nodule in the gland). In addition, the criteria used to define goiter were never defined in the studies. However, the consistency among studies, the

strength of the association, and the consistency with established causes (e.g., in all studies, ORs were increased with radiation) strongly support the hypothesis that thyroid nodules and, to a lesser degree, goiter are risk factors (potential causes) of thyroid cancer in humans. It should be pointed out, however, that in the two studies that were analyzed for

an association between hypothyroidism and thyroid cancer, neither showed a relationship (McTiernan *et al.*, 1984; Ron *et al.*, 1987).

In summary, there is considerably less support for a role for TSH in thyroid carcinogenesis in humans than in experimental animals. To the extent TSH pertains, humans appear to be less sensitive to its effects than animals.

APPENDIX A

COMBINED TREATMENT STUDIES PRODUCING THYROID TUMORS

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

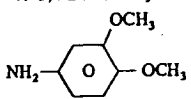
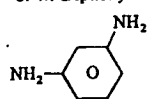
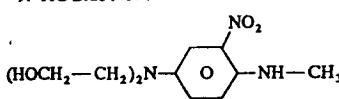
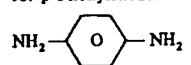
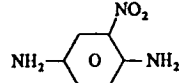
APPENDIX A—Continued

Test animal	Treatment A	Treatment B	Results	Ref.
Wistar rat (male)	DHPN (70 mg/100 g body wt given sc once/week for 4 or 6 weeks)	PB (500 ppm in diet for 12 weeks)	adenomas and increased carcinomas after 8 weeks of DHPN (no amitrole: 58% adenomas, 18% carcinomas; with amitrole: 100% adenomas, 42% carcinomas). No tumors with amitrole alone. PB after 4 weeks of DHPN-induced thyroid adenomas at 66% and carcinomas at 10%. No tumors with DHPN or PB alone. PB after 6 weeks of DHPN-accelerated development of adenomas and induced carcinomas (no PB: 23% adenomas, no carcinomas; with PB: 100% adenomas, 25% carcinomas; no tumors with PB alone).	Hiasa <i>et al.</i> (1982b)
		BB (500 ppm in diet for 12 weeks)	PB after 4 weeks of DHPN-induced thyroid adenomas (23%) but no carcinomas. No tumors with BB alone. BB after 6 weeks of DHPN-accelerated development of adenomas and induced a small number of carcinomas (no BB: 23% adenomas, no carcinomas; with BB: 45% adenomas, 10% carcinomas; no tumors with BB alone).	
			^a PB for 12 or 19 weeks after DHPN-enhanced development of thyroid adenomas. PB for 19 weeks after DHPN-induced thyroid carcinomas at 12%. Not seen with DHPN alone. PB alone produced no tumors.	
Wistar rat (male)	DHPN (single sc dose of 280 mg/100 g body wt)	PB (500 ppm in diet for 6, 12, or 19 weeks)		Hiasa <i>et al.</i> (1983)
Wistar rat (male)	DHPN (single sc dose of 280 mg/100 g body wt)	PTU (1500 ppm in diet for 19 weeks)	PTU after DHPN-enhanced development	Kitahori <i>et al.</i> (1984)

APPENDIX A—Continued

Test animal	Treatment A	Treatment B	Results	Ref.
Wistar rat (male)	DHPN (single ip dose of 280 mg/100 g body wt)	MDA (1000 ppm in diet for 19 weeks)	of thyroid follicular cell adenomas and induced carcinomas (no PTU: 19% adenomas, 0% carcinomas; with PTU: 100% adenomas, 52% carcinomas). PTU alone produced no tumors.	Hiasa <i>et al.</i> (1984)
F344/NCr rat (male)	NMU (single iv dose of 41.2 mg/kg body wt)	Iodine-deficient diet after 2 weeks until 20 or 33 week	Iodine deficiency after NMU-enhanced development of thyroid follicular cell adenomas and carcinomas (NMU alone: 10% adenomas at 20 weeks and 70% adenomas at 33 weeks, 10% carcinomas at 33 weeks; NMU with iodine deficiency: 100% adenomas at 20 weeks and 100% carcinomas at 33 weeks; no tumors following iodine deficiency alone).	Ohshima and Ward (1986)
F344/NCr rat (male)	NMU (single iv dose of 41.2 mg/kg body wt)	Iodine deficiency after 2 weeks until 52 and 77 week	Iodine deficiency after NMU-enhanced development of the thyroid follicular cell carcinomas (NMU alone: 32% carcinomas at 52 weeks; NMU with iodine deficiency: 90% at 52 weeks). Iodine deficiency alone induced mostly thyroid adenomas and a few carcinomas (40% adenomas at 52 weeks, 60% adenomas at 77 weeks, and 10% carcinomas at 77 weeks).	Ohshima and Ward (1984)

APPENDIX B—Continued

	Thyroid tumors				Other tumors			
	Rat		Mouse		Rat		Mouse	
	M	F	M	F	M	F	M	F
7. 3,4-Dimethoxyaniline 	-	-	-	-	-	-	-	-
8. <i>m</i> -Diphenylenediamine 	-	-	-	-	-	-	-	-
9. HC Blue No. 1 	-	-	+	-	Liver	Lung	Liver	Liver
10. <i>p</i> -Phenylenediamine 	-	-	-	-	-	-	-	-
11. 2-Nitro- <i>p</i> -phenylenediamine 	-	-	-	-	-	-	-	-

APPENDIX C

GENOTOXICITY: ETHYLENE THIOUREA

	Reported effect	Ref.
1. Gene mutations		
A. Bacteria		
<i>Salmonella</i> (Ames)		
G46	w	Seiler (1974)
G46		
<i>N</i> -nitrosoethylenethiourea	+	Seiler (1977)
Multiple strains		
(-NO ₂)	w	Shirasu <i>et al.</i> (1977)
(+NO ₂)	+	
Mouse/rat host mediated G46		
(-NO ₂)	-	
(+NO ₂)	+	
Multiple strains	+ TA 1530 only	Schupbach and Hummler (1977)

APPENDIX C—Continued

	Reported effect	Ref.
Mouse host mediated G46, TA 1530	+ TA 1530 only	
Multiple strains	+ in all	Anderson and Styles (1978)
TA 1950		
(-NO ₂)	w	Autio <i>et al.</i> (1982)
(+NO ₂)	+	
Mouse host mediated (TA 1950)		
(-NO ₂)	w	
(+NO ₂)	+	
Multiple strains	w TA 1535 only	Moriya <i>et al.</i> (1983)
Mouse host mediated (TA 1950)		
(-NO ₂)	-	Braun <i>et al.</i> (1977)
(+NO ₂)	+	
Multiple strains/replications in different labs	w TA 1535 - all others	Mortelmans <i>et al.</i> (1986)
Multiple strains/replications in different labs	-	Bridges <i>et al.</i> (1981)
<i>E. coli</i>		
WP2		
(-NO ₂)	-	Shirasu <i>et al.</i> (1977)
(+NO ₂)	+	
WP2	-	
B. Eukaryotic microorganisms		
<i>Saccharomyces</i> (XV 185-14C)	+ requires S9	Mehta and von Borstel (1981)
<i>Schizosaccharomyces</i>	-	Loprieno (1981)
C. Higher eukaryotes		
Mouse lymphoma cells (TK)	-	Jotz and Mitchel (1981)
Mouse lymphoma cells	+	NTP (1986)
Chinese hamster ovary (several loci)	-	Carver <i>et al.</i> (1981)
<i>Drosophila</i> XLRL	-	Valencia and Houtchens (1981)
<i>Drosophila</i> XLRL	- injection	Woodruff <i>et al.</i> (1985)
	? feeding	
<i>Drosophila</i> XLRL	+	NTP (1986)
2. Chromosome effects		
A. Numerical aberrations		
<i>Saccharomyces</i> mitotic aneuploidy	+	Parry and Sharp (1981)
Mouse micronucleus (see B, below)		
B. Structural aberrations		
Chinese hamster ovary cells	-	Shirasu <i>et al.</i> (1977)
Chinese hamster ovary cells	-	Nastaranjan and van Kesteren-van Leeuwen (1981)
Chinese hamster ovary cells	-	NTP (1986)
Mouse micronucleus (B6C3F1)	-	Salamone <i>et al.</i> (1981)
Mouse micronucleus (ICR)	-	Kirkhart (1981)
Mouse micronucleus (CD-1)	-	Tsuchimoto and Matter (1981)
Mouse micronucleus		
(-NaNO ₂)	-	Seiler (1975)
(+NaNON ₂)	+	
Mouse micronucleus	-	Schupbach and Hummler (1977)
Mouse dominant lethal	-	Shirasu <i>et al.</i> (1977)
Mouse dominant lethal	-	Schupbach and Hummler (1977)
Mouse dominant lethal		
(+NaNO ₂) preimplantation loss	+	Teramoto <i>et al.</i> (1978)
postimplantation loss	-	
Chinese hamster bone marrow		

APPENDIX C—Continued

	Reported effect	Ref.
(+NaNO ₂)	+	Seiler (1977)
Rat bone marrow	—	Shirasu <i>et al.</i> (1977)
<i>Drosophila</i> reciprocal translocation	—	NTP (1986)
C. Sister chromatid exchanges		
Chinese hamster ovary cells	—	Evans and Mitchel (1981)
Chinese hamster ovary cells	—	Nastaranjan and van Kesteren-van Leeuwen (1981)
Chinese hamster ovary cells	—	Perry and Thomson (1981)
Chinese hamster ovary cells	—	NTP (1986)
Mouse <i>in vivo</i> (CBA/J)	—	Paika <i>et al.</i> (1981)
3. DNA damage		
<i>B. subtilis</i> (rec)	w without S9 — with S9	Kada (1981)
<i>E. coli</i> (pol A)	—	Green (1981)
<i>E. coli</i> (rec)	+ with S9	Ichinotsubo <i>et al.</i> (1981)
<i>E. coli</i> (rec, pol A)	—	Tweats (1981)
<i>E. coli</i> (pol A)	w without S9 — with S9	Rosenkranz <i>et al.</i> (1981)
<i>E. coli</i> (lambda induction)	+	Thomson (1981)
<i>Saccharomyces</i> mitotic cross-over	—	Kassinova <i>et al.</i> (1981)
<i>Saccharomyces</i> mitotic gene conversion	—	Jagannath <i>et al.</i> (1981)
<i>Saccharomyces</i> mitotic gene conversion	—	Zimmernann and Scheel (1981)
<i>Saccharomyces</i> (JDI) mitotic gene conversion	+ without S9	Sharp and Perry (1981a)
<i>Saccharomyces</i> (RAD) differential growth	+	Sharp and Perry (1981b)
Unscheduled DNA synthesis WI-38 cells	—	Robinson and Mitchell (1981)
Human fibroblasts	—	Agrelo and Amos (1981)
Mouse sperm morphology	—	Wyrobek <i>et al.</i> (1981)
Mouse sperm morphology	—	Tophan (1980)
4. <i>In vitro</i> transformation		
Baby hamster kidney (BHK 21)	+	Daniel and Dehnel (1981)
Baby hamster kidney (BHK 21)	+	Styles (1981)
Syrian hamster embryo, adenovirus infected (SHE-SA7)	—	Hatch <i>et al.</i> (1986)

Note. +, positive; w, weak positive; ?, equivocal; —, negative.

APPENDIX D

GENOTOXICITY: 4,4'-OXYDIANILINE

	Reported effect	Ref.
1. Gene mutation		
A. Bacteria		
<i>Salmonella</i> (Ames)		
TA 98	+ requires S9	Lavoie <i>et al.</i> (1979)
TA 100	+ assayed only in presence of S9	
TA 98	w requires S9	Parodi <i>et al.</i> (1981)
TA 100	+ requires S9	
TA 98	+ requires S9	Tanaka <i>et al.</i> (1985)

APPENDIX D—Continued

	Reported effect	Ref.
TA 100	+ requires S9	
TA 97	+ requires S9	NTP (1987)
TA 98	+ requires S9	(personal communication E. Zeiger)
TA 100	+ with or without S9	
TA 1535	+ requires hamster S9	
TA 1537	+ assayed only with S9; requires hamster S9	
■ Eukaryotes		
Mammalian cells in culture		
Mouse lymphoma	+	NTP (1986)
2. Chromosome effects		
Chinese hamster ovary cells		
Structural chromosome aberrations	+	NTP (1986)
Sister chromatid exchanges	+	
Rat bone marrow		
Sister chromatid exchanges	—	Parodi <i>et al.</i> (1983)
3. DNA damage		
Unscheduled DNA synthesis (rat hepatocytes)		
<i>In vivo</i>	—	Mirsalis <i>et al.</i> (1983)
<i>In vitro</i>	—	
4. <i>In vitro</i> transformation		
Syrian hamster embryo cells	?	Tu <i>et al.</i> (1986)
Enhancement of virus-infected transformation of Syrian hamster embryo cells	+	Hatch <i>et al.</i> (1986)

Note. +, positive; w, weak positive; ?, equivocal; —, negative.

APPENDIX E

GENOTOXICITY: AMITROLE

	Reported effect	Ref.
1. Gene mutations		
A. Bacteria		
<i>Salmonella</i> (Ames)	—	See multiple bacterial tests summarized in Bridges <i>et al.</i> (1981)
	—	McCann and Ames (1976)
TA 1950, mouse host mediated (—NO ₂)	—	Braun <i>et al.</i> (1977)
(+NO ₂)	w	
	—	Dunkel (1979)
	—	Rosenkranz and Poirier (1979)
	—	Moriya <i>et al.</i> (1983)
	—	NTP (1986)

APPENDIX E—Continued

	Reported effect	Ref.
<i>E. coli</i>		
WP2uvrA (P)	+	Venitt and Crofton-Sleigh (1981)
WP2uvrA	-	Matsushima <i>et al.</i> (1981)
WP2uvrA/pKM101	-	Matsushima <i>et al.</i> (1981)
<i>Streptomyces</i>	w	Carere <i>et al.</i> (1978)
B. Eukaryotic microorganisms		
<i>Saccharomyces</i> (RV)	-	Mehta and von Borstel (1981)
C. Higher eukaryotes		
<i>Drosophila</i> XLRL	-	Laamanen <i>et al.</i> (1976)
	-	Vogel <i>et al.</i> (1980)
	-	Vogel <i>et al.</i> (1981)
	?	NTP (1986)
	feeding, ?;	Woodruff <i>et al.</i> (1985)
	injection, -	
	-/-/-	NTP (1986)
Mouse lymphoma L5178Y cells (TK)		
Syrian hamster embryo cells		
Ouabain	+	Tsutsui <i>et al.</i> (1984)
6-Thioguanine	+	Tsutsui <i>et al.</i> (1984)
2. Chromosome effects		
A. Numerical aberrations		
<i>Saccharomyces</i> (D6)	-	Parry and Sharp (1981)
<i>Aspergillus</i> mitotic nondisjunction	w	Bignami <i>et al.</i> (1977)
<i>Drosophila</i> sex chromosome nondisjunction	-	Laamanen <i>et al.</i> (1976)
B. Structural aberrations		
Human lymphocytes <i>in vitro</i>	-	Meretoja <i>et al.</i> (1976)
Mouse micronucleus (B6C3F1) (CD-1)	-	Salomone <i>et al.</i> (1981)
	-	Tsuchimoto and Matter (1981)
Mouse dominant lethal (Ha, I CR)	-	Food and Drug Research (1978)
C. Other effects		
Sister chromatid exchange		
CHO	+	Perry and Thomson (1981)
	+	NTP (1986)
CHO	+	
3. DNA damage		
<i>Bacillus subtilis</i>		
Rec	+	Kada (1981)
<i>E. coli</i>		
Rec	-	Green (1981)
Rec	-	Ichinotsubo <i>et al.</i> (1981)
	-	Mamber <i>et al.</i> (1983)
Rec	-	Tweats (1981)
Rec	-	Rosenkranz <i>et al.</i> (1981)
PoA	-	

APPENDIX E—Continued

	Reported effect	Ref.
Lambda prophage induction	-	Thomson (1981)
<i>Saccharomyces cerevisiae</i>		
(D3) mitotic crossover	-	Simmon (1979)
(race X11) mitotic crossover	-	Kassinova <i>et al.</i> (1981)
(D4) mitotic gene conversion	-	Jagannath <i>et al.</i> (1981)
(D7) mitotic gene conversion	-	Zimmerman and Scheel (1981)
(JD1) mitotic gene conversion	+	Sharp and Perry (1981, 1981a)
(RAD) cell growth	+	Sharp and Perry (1981b)
<i>Aspergillus</i> mitotic crossover	w	Bignami <i>et al.</i> (1977)
Unscheduled DNA synthesis (HeLa)	+	Martin and McDermid (1981)
MLV integration enhancement (C3H2K)	-	Yoshikur and Matsushima (1981)
Mouse sperm head abnormality	-	Tophan (1980)
4. <i>In vitro</i> transformation		
Syrian hamster embryo cells	+	Dunkel <i>et al.</i> (1981)
	+	Tsutsui <i>et al.</i> (1980)
Baby hamster kidney cells (BHK)	+	Styles (1980)
	+	Styles (1981)
	-	Daniel and Dehnel (1981)
Rat embryo cells		
Rauscher murine leukemia virus infected	+	Dunkel <i>et al.</i> (1981)
	+	NTP (1983)

Note. +, positive; w, weak positive; ?, equivocal; -, negative.

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REFERENCES

- ADAMS, J. M., HARRIS, A. W., PINKERT, C. A., CORCORAN, L. M., ALEXANDER, W. S., CORY, S., PALMITER, R. D., AND BRINSTER, R. L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature (London)* 318, 533-538.
- AGRELLO, C., AND AMOS, H. (1981). DNA repair in human fibroblasts. (1981). In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 528-532. Elsevier, NY.
- ALDERSON, M. R. (1980). Thyroid cancer epidemiology. *Recent Results Cancer Res.* 73, 1-22.
- ALLEN-ROWLANDS, C. F., CASTRACANE, V. D., HAMILTON, M. G., AND SEIFTER, J. (1981). Effect of polybrominated biphenyls (PBB) on the pituitary-thyroid axis of the rat. *Proc. Soc. Exp. Biol. Med.* 160, 506-514.
- ALVARES, A. P., BICKERS, D. R., AND KAPPAS, A. (1973). Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. *Proc. Natl. Acad. Sci. USA* 70, 1321-1325.
- ANDERSON, D., AND STYLES, J. A. (1978). Appendix II.

- The bacterial mutation test. *Brit. J. Cancer* 37, 924-930.
- ARNOLD, D. E., KREWSKI, D. R., JUNKINS, D. B., MCGUIRE, P. F., MOODIE, C. A., AND MUNRO, I. C. (1983). Reversibility of ethylenethiourea-induced thyroid lesions. *Toxicol. Appl. Pharmacol.* 67, 264-273.
- AR-RUSHDI, A., NISHIKURA, K., ERIKSON, J., WATT, R., ROVERA, G., AND CROCE, C. M. (1983). Differential expression of the translocated and the untranslocated c-myc oncogene in Burkitt lymphoma. *Science* 222, 390-393.
- ASTWOOD, E. B., BISSELL, A., AND HUGHES, A. M. (1945). Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland. *Endocrinology* 37, 456-481.
- ASTWOOD, E. B., SULLIVAN, B., BISSELL, A., AND TYSLOWITZ, R. (1943). Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. *Endocrinology* 32, 210-225.
- AUTO, K., VON WRIGHT, A., AND PYYSALO, H. (1982). The effect of oxidation of the sulfur atom on the mutagenicity of ethylene thiourea. *Mutat. Res.* 106, 27-31.
- AXELROD, A. A., AND LEBLOND, C. P. (1955). Induction of thyroid tumors in rats by a low iodine diet. *Cancer* 8, 339-367.
- BACHRACH, L. K., EGGO, M. C., MAK, W. W., AND BURROW, G. N. (1985). Phorbol esters stimulate growth and inhibit differentiation in cultured thyroid cells. *Endocrinology* 116, 1603-1609.
- BAHN, A. K., MILLS, J. L., SNYDER, P. J., GANN, P. H., HOUTEN, L., BIALIK, O., HOLLMANN, L., AND UTIGER, R. D. (1980). Hypothyroidism in workers exposed to polybrominated biphenyls. *N. Engl. J. Med.* 302, 31-33.
- BAKER, B. L., AND YU, Y.-Y. (1971). Hypophyseal changes induced by thyroid deficiency and thyroxine administration as revealed by immunohistochemical staining. *Endocrinology* 89, 996-1004.
- BALSAM, A., AND OPPENHEIMER, J. H. (1975). Pituitary tumor with primary hypothyroidism. *N.Y. State J. Med.* 75, 1737-1741.
- BARBACID, M. (1986). Oncogenes and human cancer: Cause or consequence. *Carcinogenesis* 7, 1037-1042.
- BASTOMSKY, C. H. (1973). The biliary excretion of thyroxine and its glucuronic acid conjugate in normal and Gunn rats. *Endocrinology* 92, 35-40.
- BASTOMSKY, C. H. (1974). Effects of polychlorinated biphenyl mixture (Arochlor 1254) and DDT on biliary thyroxine excretion in rats. *Endocrinology* 95, 1150-1155.
- BASTOMSKY, C. H. (1977a). Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Endocrinology* 101, 292-296.
- BASTOMSKY, C. H. (1977b). Goiters in rats fed polychlorinated biphenyls. *Canad. J. Physiol. Pharmacol.* 55, 288-292.
- BASTOMSKY, C. H., AND MURTHY, P. V. N. (1976). Enhanced in vitro hepatic glucuronidation of thyroxine in rats following cutaneous application or ingestion of polychlorinated biphenyls. *Canad. J. Physiol. Pharmacol.* 54, 23-26.
- BASTOMSKY, C. H., MURTHY, P. V. N., AND BANOVAC, K. (1976). Alterations of thyroxine metabolism produced by cutaneous application of microscopically immiscible oil: Effects due to polychlorinated biphenyls. *Endocrinology* 98, 1309-1314.
- BASTOMSKY, C. H., AND PAPAPETROU, P. D. (1973). Effect of methylcholanthrene on biliary thyroxine excretion in normal and Gunn rats. *J. Endocrinol.* 56, 267-273.
- BASTOMSKY, C. H., SOLYMOSS, B., ZSIGMOND, G., AND WYSE, J. M. (1975). On the mechanism of polychlorinated biphenyl-induced hypobilirubinaemia. *Clin. Chem. Acta.* 61, 171-174.
- BECKER, D. V. (1984). Choice of therapy for Graves' hyperthyroidism: *N. Engl. J. Med.* 311, 464-466.
- BIELSCHOWSKY, F. (1953). Chronic iodine deficiency as a cause of neoplasia in thyroid and pituitary of aged rats. *Brit. J. Cancer* 7, 203-213.
- BIELSCHOWSKY, F. (1955). Neoplasia and internal environment. *Brit. J. Cancer* 9, 80-116.
- BIELSCHOWSKY, F., AND GOODALL, C. M. (1963). A reassessment of the thyroid tumors induced by goitrogens in mice. *Proc. Univ. Otago Med. Sch.* 41, 3-4.
- BIGNAMI, M., AULICINO, F., VELCICH, A., CARERE, A., AND MORPURGO, G. (1977). Mutagenic and recombinogenic action of pesticides in *Aspergillus nidulans*. *Mutat. Res.* 46, 395-402.
- BIGOS, S. T., RIDGWAY, E. C., KOURIDES, I. A., AND MALOOF, F. (1978). Spectrum of pituitary alterations with mild and severe thyroid impairment. *J. Clin. Endocrinol. Metab.* 46, 317-325.
- BONE, E. A., ALLING, D. W., AND GROLLMAN, E. F. (1986). Norepinephrine and thyroid-stimulating hormone induce inositol phosphate accumulation in FRTL-5 cells. *Endocrinology* 119, 2193-2200.
- BOORMAN, G. A. (1983). Follicular cell hyperplasia, thyroid, rat. In *Endocrine System* (T. C. Jones, V. Mohr, and R. D. Hunt, Eds.), pp. 176-184. Springer-Verlag, Berlin.
- BORÓWSKI, G. D., GAROFANO, C. D., ROSE, L. I., SPIELMAN, S. R., ROTMENSCH, H. R., GREENSPAN, A. M., AND HOROWITZ, L. N. (1985). Effect of long-term amiodarone therapy on thyroid hormone levels and thyroid function. *Amer. J. Med.* 78, 443-450.
- BRAUN, R., SCHONEICH, J., AND ZIEBARTH, D. (1977). In vivo formation of N-nitroso compounds and detection of their mutagenic activity in the host-mediated assay. *Cancer Res.* 37, 4572-4579.
- BRIDGES, B. A., MACGREGOR, D., AND ZEIGER, E. (1981). Summary report on the performance of bacterial mutation assays. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 49-67. Elsevier, NY.
- BULL, G. M., AND FRASER, R. (1950). Myxoedema from resorcinol ointment applied to leg ulcers. *Lancet* 1, 851-855.
- BYRNE, J. J., CARBONE, J. P., AND HANSON, E. A. (1987). Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polychlorinated biphenyl. *Endocrinology* 121, 520-527.
- CARERE, A., ORTALI, V. A., CARDAMORE, G., TORRACCA, A. M., AND RASCHETTI, R. (1978). Microbiological mutagenicity studies of pesticides in vitro. *Mutat. Res.* 57, 277-286.
- CARLTON, W. W., AND GRIES, C. L. (1983). Adenoma and carcinoma, pars distalis, rat. In *Endocrine System* (V. C. Jones, V. Mohr, and R. D. Hunt, Eds.), pp. 134-144. Springer-Verlag, Berlin.
- CARVER, J. H., SALAZAR, E. P., KNIZE, M. G., AND WANDRES, D. L. (1981). Mutation induction and multiple gene loci in Chinese hamster ovary cells: The genetic activity of 15 coded carcinogens and noncarcinogens. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 594-601. Elsevier, NY.
- CHESNEY, A. M., CLAWSON, T. A., AND WEBSTER, B. (1928). Endemic goitre in rabbits: Incidence and characteristics. *Bull. Johns Hopkins Hosp.* 43, 261-277.
- CHIN, W. W., SHUPNIK, M. A., ROSS, D. S., HABENER, J. F., AND RIDGWAY, E. C. (1985). Regulation of the α - and thyrotropin β -subunit messenger ribonucleic acids by thyroid hormones. *Endocrinology* 116, 873-878.
- CHRISTOV, K. (1975). Thyroid cell proliferation in rats and induction of tumors by X-rays. *Cancer Res.* 35, 1256-1262.
- CLAYSON, D. B., AND GARNER, R. C. (1976). Carcinogenic aromatic amines and related compounds. In *Chemical Carcinogens* (C. E. Searle, Ed.), pp. 366-461. ACS Monograph 173, American Chemical Society, Washington, DC.
- COLLETTA, G., CIRAFICI, A. M., AND VECCHIO, G. (1986). Induction of the c-fos oncogene by thyrotropic hormone in rat thyroid cells in culture. *Science* 223, 458-460.
- COLLINS, W. T., JR., CAPEN, C. C., KASZA, L., CARTER, C., AND DAILEY, R. F. (1977). Effect of polychlorinated biphenyl (PCB) on the thyroid gland of rats. *Amer. J. Pathol.* 89, 119-136.
- CONNAY, A. H. (1967). Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19, 317-366.
- CONNAY, A. H. (1982). Induction of microsomal enzymes for chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes memorial lecture. *Cancer Res.* 42, 4875-4917.
- COOPER, D. S. (1984). Antithyroid drugs. *N. Engl. J. Med.* 311, 1353-1362.
- CORCORAN, J. M., WATERS, M. J., EASTMAN, C. J., AND JORGENSEN, G. (1986). Epidermal growth factor: Effect on circulating thyroid hormone levels in sheep. *Endocrinology* 119, 214-217.
- CROCE, C. M. (1986). Chromosome translocations and cancer. *Cancer Res.* 46, 6019-6023.
- CUMMINGS, S. W., AND PROUGH, R. A. (1983). Metabolic formation of toxic metabolites. In *Biological Basis of Detoxication* (J. Caldwell and W. B. Jacoby, Eds.). Academic Press, New York/London.
- DANIEL, M. R., AND DEHNEL, J. M. (1981). Cell transformation with baby hamster kidney cells. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 626-637. Elsevier, NY.
- DAVIDSON, B., SOODAK, M., NEARY, J. T., STROUT, H. V., KIEFFER, J. D., MOYER, H., AND MALOOF, F. (1978). The irreversible inactivation of thyroid peroxidase by methylmercaptoimidazole, thiouracil and propylthiouracil in vitro and its relationship to in vivo findings. *Endocrinology* 103, 871-882.
- DAVIES, T. F. (1985). Positive regulation of the guinea pig thyrotropin receptor. *Endocrinology* 117, 201-207.
- DE GROOT, L. J. (1979). Thyroid Neoplasia. In *Endocrinology* (L. J. De Groot et al., Eds.), Vol. 1, pp. 509-521. Grune and Stratton, New York.
- DE GROOT, L. J., AND STANBURY, J. B. (1975). *The Thyroid and its Diseases*, Chaps. 4, 11, and 13. Wiley, New York.
- DENEJ, J. F., HAUMONT, S., CORNETTE, C., AND BECKERS, C. (1981). Correlated functional and morphometric study of thyroid hyperplasia induced by iodine deficiency. *Endocrinology* 108, 2352-2358.
- DENT, J. N., GODSDEN, E. L., AND FURTH, J. (1956). Further studies on induction and growth of thyrotropic pituitary tumors in mice. *Cancer Res.* 16, 171-174.
- DERE, W. H., HIRAYU, H., AND RAPOPORT, B. (1985). TSH and cAMP enhance expression of the myc proto-oncogene in cultured thyroid cells. *Endocrinology* 117, 2249-2251.
- DE SERRES, F. J., AND ASHBY, J. (Eds.) (1981). *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research*. Elsevier, NY.
- DOBYNS, B. M., SHELINE, G. E., WORKMAN, J. B., THOMPSON, E. A., MCCONAHEY, W. M., AND BECKER, D. V. (1974). Malignant and benign neoplasms of the thyroid in patients treated for hyperthyroidism. A report of the cooperative thyrotoxicosis therapy follow-up study. *J. Clin. Endocrinol. Metab.* 38, 976-998.
- DOHLER, K.-D., WONG, C. C., AND VON ZUR MUHLEN, A. (1979). The rat as model for the study of drug effects on thyroid function: Consideration of methodological problems. *Pharmacol. Ther.* 5, 305-318.

- DONIACH, I. (1950). The effect of radioactive iodine alone and in combination with methylthiouracil and acetylaminofluorene upon tumour production in the rat's thyroid gland. *Brit. J. Cancer* 4, 223-234.
- DONIACH, I. (1953). The effect of radioactive iodine alone and in combination with methylthiouracil upon tumour production in the rat's thyroid gland. *Brit. J. Cancer* 7, 181-202.
- DONIACH, I. (1970a). Actiological considerations of thyroid carcinoma. In *Tumours of the Thyroid Gland* (D. Smithers, Ed.), Vol. 6, pp. 55-72. Livingstone, Edinburgh.
- DONIACH, I. (1970b). Experimental thyroid tumors. In *Tumours of the Thyroid Gland* (D. Smithers, Ed.), Vol. 6, pp. 73-199. Livingstone, Edinburgh.
- DONIACH, I. (1974). Carcinogenic effect of 100, 250, and 500 rad X-rays on the rat thyroid gland. *Brit. J. Cancer* 30, 487-495.
- DONIACH, I., AND WILLIAMS, E. D. (1962). The development of thyroid and pituitary tumors in the rat two years after partial thyroidectomy. *Brit. J. Cancer* 16, 222-231.
- DUNKEL, V. C. (1979). Collaborative studies on the Salmonella/microsome mutagenicity assay. *J. Assoc. Off. Anal. Chem.* 62, 874-882.
- DUNKEL, V. C., PIENTA, R. J., SIVAK, A., AND TRAUJLIK, A. (1981). Comparative neoplastic transformation responses of BALB/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical carcinogens. *J. Natl. Cancer Inst.* 67, 1303-1315.
- DUNN, T. B. (1975). *The Unseen Fight Against Cancer: Experimental Cancer Research: Its Importance to Human Cancer*, p. 111. Bates Publishing, Charlotte, NC.
- EOBICHON, D. J., AND COMEAU, A. M. (1974). Comparative effects of commercial Arochlors on rat liver enzyme activities. *Chem. Biol. Interact.* 9, 341-347.
- EISEN, H. G., HANNAH, R. R., LEGRAVEREND, C., OKEY, A. B., AND NEBERT, D. W. (1983). In *Biochemical Actions of Hormones* (G. Litwack, Ed.), Vol. 10, pp. 227-257. Academic Press, New York.
- ELTOM, M., SALIH, M. A. H., BOSTROM, H., AND DAHLBERG, P. A. (1985). Differences in aetiology and thyroid function in endemic goiter between rural and urban areas of the Darfur region of the Sudan. *Acta Endocrinol.* 108, 356-360.
- ENGLER, H., TAUROG, A., AND NAKASHIMA, T. (1982). Mechanism of inactivation of thyroid peroxidase by thiouretene drugs. *Biochem. Pharmacol.* 31, 3801-3806.
- ERIKSON, J., FINGER, L., SUN, L., AR-RUSHDI, A., NISHIKURA, K., MINOWADA, J., FINAN, J., EMANUEL, B. S., NOWELL, P. C., AND CROCE, C. M. (1986). De-regulation of c-myc by translocation of the α -locus of the T-cell receptor in T-cell leukemia. *Science* 232, 884-886.
- EVANS, E. L., AND MITCHELL, A. D. (1981). Effects of 20 coded chemicals on sister chromatid exchange frequency in cultured Chinese hamster ovary cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 539-550. Elsevier, NY.
- FABER, J., LUMHOLTZ, I. B., KIRKEGAARD, C., POULSEN, S., HOLME JORGENSEN, P., SIERSBAEK-NIELSEN, K., AND FRUS, T. (1985). The effects of phenytoin (diphenylhydantoin) on the extrathyroidal turnover of thyroxine, 3,5,3'-triiodothyronine, 3,3',5'-triiodothyronine and 3',5'-diodothyronine in man. *J. Clin. Endocrinol. Metab.* 61, 1093-1099.
- FIELD, J. B., DEKKER, A., TITUS, G., KERINS, M. F., WORDEN, W., AND FRUMESS, R. (1979). *In vitro* and *in vivo* refractoriness to thyrotropin stimulation of iodine organification and thyroid hormone secretion. *J. Clin. Invest.* 64, 265-271.
- FILETTI, S., BELFIORE, A., DANIELS, G. H., IPPOLITO, O., VIGNERI, R., AND INGBAR, S. (1988). The role of thyroid-stimulating antibodies of Graves' disease in differentiated thyroid cancer. *N. Engl. J. Med.* 318, 753-759.
- FINGER, L. R., HARVEY, R. C., MOORE, R. C. A., SHOWE, L. C., AND CROCE, C. M. (1986). A common mechanism of chromosomal translocation in T- and B-cell neoplasia. *Science* 234, 982-985.
- FISH, L. H., SCHWARTZ, H. L., CAVANAUGH, J., STEFFES, M. W., BANTLE, J. P., AND OPPENHEIMER, J. H. (1987). Replacement dose, metabolism, and bioavailability of *l*-*l*-thyroxine in the treatment of hypothyroidism. Role of triiodothyronine in pituitary feedback in humans. *N. Engl. J. Med.* 316, 764-770.
- Food and Drug Research. (1978). *A Study to Determine the Potential of Amitrole to Induce Dominant Lethal Mutations in Ha(ICR) Mice*. FDRL Report No. 5502.
- FREGLY, M. J., WATERS, I. W., AND STRAW, J. A. (1968). Effect of isomers of DDD on thyroid and adrenal function in rats. *Canad. J. Physiol. Pharmacol.* 45, 59-66.
- FRIEDMAN, B. A., FRACKELTON, A. R., JR., ROSS, A. H., CONNORS, J. M., FUJIKI, H., SUGIMURA, T., AND ROSNER, M. R. (1984). Tumor promoters block tyrosine-specific phosphorylation of the epidermal growth factor receptor. *Proc. Natl. Acad. Sci. USA* 81, 3034-3038.
- FRITH, C. H., AND HEATH, J. E. (1983). Adenoma, thyroid, mouse. In *Endocrine System* (T. C. Jones, V. Mohr, and R. D. Hunt, Eds.), pp. 184-191. Springer-Verlag, Berlin.
- FURTH, J. (1969). Pituitary cybernetics and neoplasia. *Harvey Lectures* 63, 47-71.
- FURTH, J., MOY, P., HERSHMAN, J. M., AND UEDA, G. (1973). Thyrotropic tumor syndrome. A multiglandular disease induced by sustained deficiency of thyroid hormones. *Arch. Pathol.* 96, 217-226.
- GALTON, V. A. (1968). The physiological role of thyroid hormone metabolism. In *Recent Advances in Endocrinology* (V. H. T. James, Ed.), 8th ed., pp. 181-206. Churchill, London.
- GEFFNER, D. L., AZUKIZAWA, M., AND HERSHMAN, J. M. (1975). Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. *J. Clin. Invest.* 55, 224-229.
- GHARIB, H., JAMES, E. M., CHARBONEAU, J. W., NAESSENS, J. M., OFFORD, K. P., AND GORMAN, C. A. (1987). Suppressive therapy with levothyroxine for solitary thyroid nodules: A double-blind controlled clinical study. *N. Engl. J. Med.* 317, 70-75.
- GILMAN, A., AND MURAD, F. (1975). Thyroid and antithyroid drugs. In *The Pharmacological Basis of Therapeutics* (L. S. Goodman and A. Gilman, Eds.), 5th ed. MacMillan Co., New York.
- GINSBERG, J., AND MURRAY, P. G. (1986). Protein kinase C activators modulate differentiated thyroid function *in vitro*. *FEBS Lett.* 206, 309-312.
- GOLDSTEIN, J. A., AND TAUROG, A. (1968). Enhanced biliary excretion of thyroxine glucuronide in rats pretreated with benzpyrene. *Biochem. Pharmacol.* 17, 1049-1056.
- GOODMAN, H. M., AND VAN MIDDLESWORTH, L. (1980). The thyroid gland. In *Medical Physiology* (V. B. Mountcastle, Ed.), Vol. 2, pp. 1495-1518. Mosby, St. Louis.
- GORBMAN, A. (1947). Thyroidal and vascular changes in mice following chronic treatment with goitrogens and carcinogens. *Cancer Res.* 7, 746-758.
- GOUSTIN, A. S., LEOF, E. B., SHIPLEY, G. D., AND MOSES, H. L. (1986). Growth factors and cancer. *Cancer Res.* 46, 1015-1029.
- GRAHAM, S. L., HANSEN, W. H., DAVIS, K. J., AND PERRY, C. J. (1973). Effects of one-year administration of ethylene thiourea upon the thyroid of the rat. *J. Agric. Food Chem.* 21, 324-329.
- GREEN, M. H. L. (1981). A differential killing test using an improved repair-deficient strain of *Escherichia coli*. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 183-194. Elsevier, NY.
- GREEN, W. L. (1978). Mechanism of action of antithyroid compounds. In *The Thyroid* (C. Werner and S. H. Ingbar, Eds.), pp. 77-87. Harper and Row, New York.
- GREER, M. A., STUDER, H., AND KENDALL, J. W. (1967). Studies on the pathogenesis of colloid goiter. *Endocrinology* 81, 623-632.
- GRIESBACH, W. E. (1941). Studies on experimental goitre. II. Changes in the anterior pituitary of the rat produced by Brassica seed diet. *Brit. J. Exp. Pathol.* 22, 245-249.
- GRIESBACH, W. E., KENNEDY, T. H., AND PURVES, H. D. (1945). Studies on experimental goiter. VI. Thyroid adenomata in rats on Brassica seed diets. *Brit. J. Exp. Pathol.* 26, 18-24.
- GROTE, W., SCHMOLDT, A., AND DAMMANN, H. G. (1975). The metabolism of foreign compounds in rats after treatment with polychlorinated biphenyls (PCBs). *Biochem. Pharmacol.* 24, 1121.
- GUTIERREZ, C., GUO, Z-S., BRUHANS, W., DEPAMPHILIS, M. L., FARREL-TOWT, J., AND JU, G. (1988). Is c-myc protein directly involved in DNA replication? *Science* 240, 1202.
- HALL, W. H. (1948). The role of initiating and promoting factors in the pathogenesis of tumours of the thyroid. *Brit. J. Cancer* 2, 273-280.
- HAMILTON, T. E., VANBELLE, G., AND LOGERFO, J. P. (1987). Thyroid neoplasia in Marshall Islanders exposed to nuclear fallout. *J. Amer. Med. Assn.* 258, 629-636.
- HAN, V. K., D'ERCOLE, A. J., AND LUND, P. K. (1987). Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science* 236, 193-197.
- HARAN-GUERA, N., PULLAR, P., AND FURTH, J. (1960). Induction of thyrotropin-dependent thyroid tumors by thyrotropes. *J. Endocrinol.* 66, 694-701.
- HASEMAN, J. K., HUFF, J., AND BOORMAN, G. A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* 12, 126-135.
- HATCH, G. G., ANDERSON, T. M., LUBET, R. A., KOURI, R. E., PUTNAM, D. L., CAMERON, J. W., NIMS, R. W., MOST, B., SPALDING, J. W., TENNANT, R. W., AND SCHECHTMAN, L. M. (1986). Chemical enhancement of SA7 virus transformation of hamster embryo cells: Evaluation by interlaboratory testing of diverse chemicals. *Environ. Mutat.* 8, 515-531.
- HAYDEN, D. W., WADE, G. G., AND HANDLER, A. H. (1978). Goitrogenic effect of 4,4'-oxydianiline in rats and mice. *Vet. Pathol.* 15, 649-662.
- HAYNES, R. C., JR., AND MURAD, F. (1985). Thyroid and antithyroid drugs. In *The Pharmacological Basis of Therapeutics* (A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad, Eds.), 7th ed., pp. 1389-1411. Macmillan, NY.
- HEDINGER, C. (1981). Geographic pathology of thyroid diseases. *Pathol. Res. Pract.* 171, 285-292.
- HEIDELBERGER, C., FREEMAN, A. E., PIENTA, R. J., SIVAK, A., BERTRAM, J. S., CASTO, B. C., DUNKEL, V. C., FRANCIS, M. W., KAKUNAGA, T., LITTLE, J. B., AND SCHECHTMAN, L. M. (1983). Cell transformation by chemical agents—A review and analysis of the literature. A report of the U.S. Environmental Protection Agency's Gene-Tox Program. *Mutat. Res.* 114, 283-385.
- HENDERSON, B. E., ROSS, R. K., PIKE, M. C., AND CASAGRANDE, J. T. (1982). Endogenous hormones as a major factor in human cancer. *Cancer Res.* 42, 3232-3239.
- HENRY, E. C., AND H. GASIEWICZ, T. A. (1987).

- Changes in thyroid hormones and thyroid glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 89, 165-174.
- HERCUS, C. E., AND PURVES, H. D. (1936). Studies on experimental and endemic goiter. *J. Hyg.* 36, 182-203.
- HIASA, Y., OHSHIMA, M., KITAHORI, Y., YUASHA, T., FUJITA, T., AND IWATA, C. (1982a). Promoting effects of 3-amino-1,2,4-triazole on the development of thyroid tumors in rats with *N*-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 3, 381-384.
- HIASA, Y., KITAHORI, Y., OHSHIMA, M., FUJITA, T., YUASHA, T., KONISHI, N., AND MIYASHIRO, A. (1982b). Promoting effects of phenobarbital and barbital on development of thyroid tumors in rats treated with *N*-bis(2-hydroxypropyl)nitrosamine. *Carcinogenesis* 3, 1187-1190.
- HIASA, Y., KITAHORI, Y., KONISHI, N., ENOKI, N., AND FUJITA, T. (1983). Effect of varying the duration of exposure to phenobarbital on its enhancement of *N*-bis(2-hydroxypropyl)nitrosamine-induced thyroid tumorigenesis in male Wistar rats. *Carcinogenesis* 4, 935-937.
- HIASA, Y., KITAHORI, Y., ENOKI, N., KONISHI, N., AND SHIMOYAMA, T. (1984). 4,4'-Diaminodiphenylmethane: Promoting effect on the development of thyroid tumors in rats treated with *N*-bis(2-hydroxypropyl)nitrosamine. *J. Natl. Cancer Inst.* 72, 471-476.
- HINKLE, P. M., AND GOH, K. B. C. (1982). Regulation of thyrotropin-releasing hormone receptors and responses by L-triiodothyronine in dispersed rat pituitary cell cultures. *Endocrinology* 110, 1725.
- HOUK, J. C. (1980). Homeostasis and control principles. In *Medical Physiology* (V. B. Mountcastle, Ed.), Vol. 1, 246-267. Mosby, St. Louis.
- HUNTON, R. B., WELLS, M. V., AND SKIPPER, E. W. (1965). Hypothyroidism in diabetics treated with sulphonylurea. *Lancet* 449-451.
- HURST, J. G., NEWCOMER, W. S., AND MORRISON, J. A. (1974). Some effects of DDT, toxaphene and polychlorinated biphenyl on thyroid function in bobwhite quail. *Poult. Sci.* 53, 125-133.
- HWANG, S. W. (1973). Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the biliary excretion of indocyanine green in rat. *Environ. Health Perspect.* 5, 227-231.
- IARC (International Agency for Research on Cancer). (1974). *IARC Monographs on the evaluation of carcinogenic risk of chemicals to man. Some antithyroid and related substances nitrofurans and industrial chemicals*, Vol. 7. WHO, IARC, Lyon, France.
- ICHINOTSUBO, D., MOWER, H., AND MANDEL, M. (1981). Testing of a series of paired compounds (carcinogenic and noncarcinogenic structural analogue) by DNA repair-deficient *E. coli* strains. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 195-198. Elsevier, NY.
- INGBAR, S. H., AND WOEBER, K. A. (1981). The thyroid gland. In *Textbook of Endocrinology* (R. H. Williams, Ed.), 6th ed., pp. 117-247. Saunders, Philadelphia.
- JACOBSEN, M. M., LEVIN, W., AND CONNEY, A. H. (1975). Studies on bilirubin and steroid glucuronidation by rat liver microsomes. *Biochem. Pharmacol.* 24, 655-665.
- JAGANNATH, D. R., VULTAGGIO, D. M., AND BRUSICK, D. J. (1981). Genetic activity of 42 coded compounds in the mitotic gene conversion assay using *Saccharomyces cerevisiae* strain D4. In *Evaluation of Short Term Tests for Carcinogens*, Vol. 1, *Progress in Mutation Research* (F. J. de Serres and J. Ashby, Eds.), pp. 456-467. Elsevier, NY.
- JAPUNDZIC, M. M. (1969). The goitrogenic effect of phenobarbital-Na on the rat thyroid. *Acta Anat.* 74, 88-96.
- JEFFERIES, D. J., AND FRENCH, M. C. (1969). Avian thyroid: Effect of *p,p'*-DDT on size and activity. *Science* 166, 1278-1280.
- JEFFERIES, D. J., AND FRENCH, M. C. (1972). Changes induced in pigeon thyroid by *p,p'*-DDE and dieldrin. *J. Wildl. Manage.* 36, 24-30.
- JEMEC, B. (1980). Studies of the goitrogenic and tumorigenic effect of two goitrogens in combination with hypophysectomy or thyroid hormone treatment. *Cancer* 45, 2138-2148.
- JOTZ, M. M., AND MITCHELL, A. D. (1981). Effects of 20 coded chemicals on the forward mutation frequency at the thymidine kinase locus in L5178Y mouse lymphoma cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 580-593. Elsevier, NY.
- KADA, T. (1981). The DNA damaging activity of 42 coded compounds in the rec-assay. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 175-182. Elsevier, NY.
- KAIBUCHI, K., TSUDA, T., KIKUCHI, A., TANIMOTO, T., YAMASHITA, T., AND TAKAI, Y. (1986). Possible involvement of protein kinase C and calcium ion in growth factor-induced expression of c-myc oncogene in Swiss 3T3 fibroblasts. *J. Biol. Chem.* 261, 1187-1192.
- KASAI, K., AND FIELD, J. B. (1982). Properties of enzyme activities involved in protein phosphorylation-dephosphorylation of thyroid plasma membranes. *Biochim. Biophys. Acta* 718, 125-134.
- KASSINOVA, G. V., KOVALTSOVA, S. V., MARFIN, S. V., AND ZAKHAROV, I. A. (1981). Activity of 40 coded compounds in differential inhibition and mitotic crossing-over assays in yeast. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 434-455. Elsevier, NY.
- KASZA, L., COLLINS, W. T., CAPEN, C. C., GARTHOFF, L. H., AND FRIEDMAN, L. (1978). Comparative toxicity of polychlorinated biphenyl and polybrominated biphenyl in the rat thyroid gland: Light and electron microscopic alterations after subacute dietary exposure. *J. Environ. Pathol. Toxicol.* 1, 587-599.
- KATZ, M. S., GREGERMAN, R. I., HORVATH, E., KOVACS, K., AND EZRIN, C. (1980). Thyrotroph cell adenoma of the human pituitary gland associated with primary hypothyroidism: Clinical and morphological features. *Acta Endocrinol.* 85, 41-48.
- KENNEDY, T. H., AND PURVES, H. D. (1941). Studies on experimental goitre. I. The effects of Brassica seed diets on rats. *Brit. J. Exp. Pathol.* 22, 241-247.
- KIMBROUGH, R. D. (1974). The toxicity of polychlorinated polycyclic compounds and related chemicals. *Crit. Rev. Toxicol.* 2, 445-498.
- KIRKHAART, B. (1981). Micronucleus test on 21 compounds. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 698-704. Elsevier, NY.
- KITAHORI, Y., HIASA, Y., KONISHI, N., ENOKI, N., SHIMOYAMA, T., AND MIYASHIRO, A. (1984). Effect of propylthiouracil on the thyroid tumorigenesis induced by *N*-bis(2-hydroxypropyl) nitrosamine in rats. *Carcinogenesis* 5, 657-660.
- KRUJER, W., COOPER, J. A., HUNTER, T., AND VERMA, I. M. (1984). Platelet-derived growth factor induces rapid but transient expression of the c-fos gene and protein. *Nature (London)* 312, 711-716.
- LAAMANEN, I., SORSA, M., BAMFORD, D., GRIPENBERG, U., AND MERETOJA, T. (1976). Mutagenicity and toxicity of amitrole. I. *Drosophila* tests. *Mutat. Res.* 40, 185-190.
- LANE, R. J. M., CLARK, F., AND MCCOLLUM, J. K. (1977). Oxyphebutazone-induced goitre. *Postgrad. Med. J.* 53, 93-95.
- LANGDON, W. Y., HARRIS, A. W., CORY, S., AND ADAMS, J. M. (1986). The c-myc oncogene perturbs B lymphocyte development in *E μ -myc* transgenic mice. *Cell* 47, 11-18.
- LARSEN, P. R. (1982a). Thyroid-pituitary interaction. *N. Engl. J. Med.* 306, 23-32.
- LARSEN, P. R. (1982b). The thyroid. In *Cecil Textbook of Medicine* (J. B. Wyngaarden and L. H. Smith, Eds.), 16th ed. Saunders, Philadelphia.
- LAVENHAR, S. R., AND MACZKA, C. A. (1985). Structure-activity consideration in risk assessment: A simulation study. *Toxicol. Indust. Health* 1, 249-259.
- LAVOIE, E., TULLEY, L., FORO, E., AND HOFFMAN, D. (1979). Mutagenicity of aminophenyl and nitrophenyl ethers, sulfides, and disulfides. *Mutat. Res.* 67, 123-131.
- LEOF, E. B., WHARTON, W., VAN WYK, J. J., AND PLEDGER, W. J. (1982). Epidermal growth factor (EGF) and somatomedin C regulate G1 progression in competent BALB/c-3T3 cells. *Exp. Cell Res.* 141, 107-115.
- LOPRIENO, N. (1981). Screening of coded carcinogenic/noncarcinogenic chemicals by a forward mutation system with the yeast *Schizosaccharomyces pombe*. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 424-433. Elsevier, NY.
- LU, A. Y. H., AND WEST, S. B. (1978). Reconstituted mammalian mixed-function oxidases: Requirements, specifications, and other properties. *Pharmacol. Ther.* 12, 337-358.
- LU, A. Y. H., AND WEST, S. B. (1980). Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol. Rev.* 31, 277-295.
- LUCIER, G. W., MCDANIEL, O. S., AND HOOK, G. E. R. (1975). Nature of the enhancement of hepatic uridine diphosphate glucuronyl transferase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Biochem. Pharmacol.* 24, 325-332.
- MACKENZIE, C. G., AND MACKENZIE, J. B. (1943). Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. *Endocrinology* 32, 185-209.
- MACKENZIE, J. B., MACKENZIE, C. G., AND MCCOLLUM, E. V. (1941). Effect of sulfanilylguanidine on thyroid of the rat. *Science* 94, 518-519.
- MEMBER, S. W., BRYSON, V., AND KATZ, S. E. (1983). The *Escherichia coli* WP2/WP100 rec assay for detection of potential chemical carcinogens. *Mutat. Res.* 119, 135-144.
- MANNERING, G. J. (1971). *Fundamentals of Drug Metabolism and Drug Disposition* (B. N. LaDu, H. G. Mandel, and E. L. Way, Eds.). Williams and Wilkins, Baltimore.
- MARTIN, C. N., AND McDERMID, A. C. (1981). Testing of 42 coded chemicals for their ability to induce unscheduled DNA repair synthesis in HeLa cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 532-537. Elsevier, NY.
- MARTINO, E., SAFRAN, M., AGHINI-LOMBARDI, F., et al. (1984). Environmental iodine intake and thyroid dysfunction during chronic amiodarone therapy. *Ann. Intern. Med.* 101, 28-34.
- MATSUSHIMA, T., TAKAMOTO, Y., SHIRAI, A., SAWAMURA, M., AND SUGIMURA, T. (1981). Reverse mutation test on 42 coded compounds with the *E. coli* WP2 system. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 387-395. Elsevier, NY.
- MCCANN, J., AND AMES, B. A. (1976). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals: Discussion. *Proc. Natl. Acad. Sci. USA* 72, 5135-5139.
- MCGINTY, D. A., AND BYWATER, W. G. (1945a). Antithyroid studies. I. The goitrogenic activity of some thioureas, pyrimidines and miscellaneous compounds. *J. Pharmacol. Exp. Ther.* 84, 342-357.
- MCGINTY, D. A., AND BYWATER, W. G. (1945b). Antithyroid studies. III. The goitrogenic activity of certain

- chemotherapeutically active sulfones and related compounds. *J. Pharmacol. Exp. Ther.* 85, 129-139.
- McKINNEY, J. D., CHAE, K., JORDAN, S., LUSTER, M., AND TUCKER, A. (1985a). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) binds the nuclear receptor for thyroxine. *Toxicologist* 5, 201.
- McKINNEY, J. D., FAWKES, J., JORDAN, S., CHAE, K., OATLEY, S., COLEMAN, R. E., AND BRINER, W. (1985b). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as a potent and persistent thyroxine agonist: A mechanistic model for toxicity based on molecular reactivity. *Environ. Health Perspect.* 61, 41-53.
- McTIERNAN, A. M., WEISS, N. S., AND DALLING, J. R. (1984). Incidence of thyroid cancer in women in relation to previous exposure to radiation therapy and history of thyroid disease. *J. Natl. Cancer Inst.* 73, 575-581.
- MEHTA, R. D., AND VON BORSTEL, R. C. (1981). Mutagenic activity of 42 encoded compounds in the haploid yeast reversion assay, strain XV 185-14. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 414-423. Elsevier, NY.
- MERETOJA, T., et al. (1976). Mutagenicity and toxicity of amitrole. II. Human lymphocyte culture tests. *Mutat. Res.* 40, 191-196.
- MEYERS, F. H., JAWETZ, E., AND GOLDFIEN, A. (1976). Thyroid and antithyroid drugs. In *Review of Medical Pharmacology*, 5th ed. Lange Medical Publishers, Los Altos, CA.
- MILMORE, J. E., CHANDRASKARAN, V., AND WEISBURGER, J. H. (1982). Effects of hypothyroidism on development of nitrosomethylurea-induced tumors of the mammary gland, thyroid gland and other tissues. *Proc. Soc. Exp. Biol. Med.* 169, 487-493.
- MIRSALIS, J., TYSON, K., BECK, J., LOH, E., STEINMETZ, K., CONTRERAS, C., AUSTERE, L., MARTIN, S., AND SPALDING, J. (1983). Induction of unscheduled DNA syntheses (UDS) in hepatocytes following *in vitro* and *in vivo* treatment. *Environ. Mutat.* 5, 482. [Abstract]
- MONEY, W. L., AND RAWSON, R. W. (1950). The experimental production of thyroid tumors in the rat exposed to prolonged treatment with thiouracil. *Cancer* 3, 321-335.
- MORIYA, M., OHTA, T., WATANABE, K., MIYAZAWA, T., KATO, K., AND SHIRASU, Y. (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat. Res.* 116, 185-216.
- MORTELMANS, K., HAWORTH, S., LAWLOR, T., SPECK, W., TAINER, B., AND ZEIGER, E. (1986). *Salmonella* mutagenicity tests. II. Results from the testing of 270 chemicals. *Environ. Mutat.* 8, Suppl. 7, 1-119.
- MORTENSEN, J. D., WOOLNER, L. B., AND BENNETT, W. A. (1955). Gross and microscopic findings in clinically normal thyroid glands. *J. Clin. Endocrinol.* 15, 1270-1280.
- MOULDING, T., AND FRASER, R. (1970). Hypothyroidism related to ethionamide. *Amer. Rev. Respir. Dis.* 101, 90-94.
- MULLER, R., BRAVO, R., AND BURCKHARDT, J. (1984). Induction of *c-fos* gene and protein by growth factors precedes activation of *c-myc*. *Nature (London)* 312, 716-720.
- MURTHY, A. S., RUSSFIELD, A. B., AND SNOW, G. J. (1985). Effect of 4,4'-oxydianiline on the thyroid and pituitary glands of F344 rats: A morphologic study with the use of the immunoperoxidase method. *J. Natl. Cancer Inst.* 74, 203-208.
- NADLER, N. J., MANDAVIA, M., AND GOLDBERG, M. (1970). The effect of hypophysectomy on the experimental production of rat thyroid neoplasms. *Cancer Res.* 30, 1909-1911.
- NASTARANJAN, A. T., AND VAN KESTEREN-VAN LEEUWEN, A. C. (1981). Mutagenic activity of 20 coded compounds in chromosome aberration/sister chromatid exchange assay using Chinese hamster ovary (CHO) cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 551-559. Elsevier, NY.
- NAS. (1980). *The Effects on Populations of Exposure to Low Levels of Ionizing Radiation*. National Research Council, National Academy of Sciences, Washington, DC.
- NCI. (1988). *Annual Cancer Statistics Review Including Cancer Trends: 1950-1985*. NIH Publication No. 88-2789, U.S. Department of Health and Human Services, National Cancer Institute, Bethesda, MD.
- NCRP. (1985). *Induction of Thyroid Cancer by Ionizing Radiation*. NCRP Report No. 80, National Council on Radiation Protection and Measurement, Bethesda, MD.
- NEWMAN, W. C., FERNANDEZ, R. C., SLAYDEN, R. M., AND MOON, R. C. (1971). Accelerated biliary thyroxine excretion in rats treated with 3-methylcholanthrene. *Proc. Soc. Exp. Biol. Med.* 138, 899-904.
- NISHIKURA, K., AR-RUSHDI, A., ERIKSON, J., WATT, R., ROVERA, G., AND CROCE, C. M. (1983). Differential expression of the normal and the translocated human *c-myc* oncogenes in B cells. *Proc. Natl. Acad. Sci. USA* 80, 4822-4826.
- NISHIZUKA, Y. (1986). Studies and perspectives of protein kinase C. *Science* 233, 305-312.
- NORRIS, J. M., KOCIBA, R. J., SCHWETZ, B. A., ROSE, J.-Q., HUMISTON, C. G., JEWETT, G. L., GEHRING, P. J., AND MAILHES, J. B. (1975). Toxicology of octabromobiphenyl and decabromodiphenyl oxide. *Environ. Health Perspect.* 11, 153-161.
- NOWELL, P. C. (1986). Mechanisms of tumor progression. *Cancer Res.* 46, 2203-2207.
- NTP. (1983). *NTP Technical Bulletin*, No. 9, p. 7. National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- NTP. (1984). *National Toxicology Program*. p. 8. Fiscal year 1984 annual plan, U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, NC.
- NTP. (1986). *CHEMTRACK National Toxicology Program*—National Institute of Environmental Health Sciences, U.S. Department of Health and Human Services, Research Triangle Park, NC.
- OHNHAUS, E. E., AND STUDER, H. (1983). A link between liver microsomal enzyme activity and thyroid hormone metabolism in man. *Brit. J. Clin. Pharmacol.* 15, 71-76.
- OHSHIMA, M., AND WARD, J. M. (1984). Promotion of *N*-methyl-*N*-nitrosourea-induced thyroid tumors by iodine deficiency in F344/NCr rats. *J. Natl. Cancer Inst.* 73, 289-296.
- OHSHIMA, M., AND WARD, J. M. (1986). Dietary iodine deficiency as a promoter and carcinogen in male F344/NCr rats. *Cancer Res.* 46, 877-883.
- OPPENHEIMER, J. H., BERNSTEIN, G., AND SURKS, M. I. (1968). Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J. Clin. Invest.* 47, 1399-1406.
- OPPENHEIMER, J. H., SHAPIRO, H. C., SCHWARTZ, H. L., AND SURKS, M. I. (1971). Dissociation between thyroxine metabolism and hormonal action in phenobarbital-treated rats. *Endocrinology* 88, 115-119.
- OPPENHEIMER, J. H. (1979). Thyroid hormone action at the cellular level. *Science* 203, 971-979.
- ORAMURA, R. Y., AND TAKAYAMA, S. (1983). Histochemical identification of hormones in pituitary tumors, rat. In *Endocrine System* (T. C. Jones, U. Mohr, and R. D. Hunt, Eds.), pp. 130-134. Springer-Verlag, New York.
- ORRICO, C., AND MYANT, N. N. (1963). Effect of salicylate on the biliary excretion of thyroxine in the rat. *Endocrinology* 72, 253-257.
- OSTP, Office of Science and Technology Policy. (1985). *Chemical carcinogens: A review of the science and its associated principles*. *Fed. Regist.* 50, 10371-10442.
- PANKA, I. J., BEAUCHESNE, M. T., RANDALL, M., SCHRECK, R. R., AND LATT, S. A. (1981). *In vivo* SCE analysis of 20 coded compounds. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 673-681. Elsevier, NY.
- PARODI, S., TANINGHER, M., BRUGO, P., PALA, M., TAMARO, M., AND MONTI-BRAGADIR, C. (1981). DNA-damaging activity *in vivo* and bacterial mutagenicity of sixteen aromatic amines and azo-derivatives, as related quantitatively to their carcinogenicity. *Carcinogenesis* 2, 1317-1326.
- PARODI, S., ZUNINO, A., OTTAGGIO, L., DEFERRARI, M., AND SANTI, L. (1983). Lack of correlation between the capability of inducing sister-chromatid exchanges *in vivo* and carcinogenic potency for 16 aromatic amines and azo derivatives. *Mutat. Res.* 108, 225-238.
- PARRY, J. M., AND SHARP, D. C. (1981). Induction of mitotic aneuploidy in the yeast strain D6 by 42 coded compounds. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 468-480. Elsevier, NY.
- PAYNTER, O. E., BURIN, G. J., JAEGER, R. B., AND GREGARIO, C. A. (1986). *Neoplasia Induced by Inhibition of Thyroid Gland Function (Guidance for Analysis and Evaluation)*. Hazard Evaluation Division, U.S. Environmental Protection Agency, Washington, DC.
- PAYNTER, O. E., BURIN, G. J., JAEGER, R. B., AND GREGARIO, C. A. (1988). Goitrogens and thyroid follicular cell neoplasia. Evidence for a threshold process. *Regul. Toxicol. Pharmacol.* 8, 102-119.
- PAZDERNIK, T. L., AND ROZMAN, K. K. (1985). Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin immunotoxicity. *Life Sci.* 36, 695-703.
- Peer Review Panel. (1987). *An Inquiry into the Mechanism of Carcinogenic Action of FD&C Red No. 3 and Its Significance for Risk Assessment*. A report by the FD&C Red No. 3 Peer Review Panel, Food and Drug Administration, Washington, DC.
- PENDERGRAST, W. J., MILMORE, B. K., AND MARCUS, S. C. (1961). Thyroid cancer and thyrotoxicosis in the United States and their relation to endemic goiter. *J. Chronic Dis.* 13, 22-38.
- PERRY, P. E., AND THOMSON, E. J. (1981). Evaluation of the sister chromatid exchange method in mammalian cells as a screening system for carcinogens. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 560-569. Elsevier, NY.
- PHILP, J. R., CROOKS, J., MACGREGOR, A. G., AND MCINTOSH, J. A. R. (1969). The growth curve of the rat thyroid under a goitrogenic stimulus. *Brit. J. Cancer* 23, 515-523.
- POLAND, A., AND GLOVER, E. (1974). Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* 10, 349-359.
- POLAND, A., AND KNUTSEI, J. C. (1982). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Ann. Rev. Pharmacol. Toxicol.* 22, 517-554.
- POLYCHRONAKOS, C., GUYDA, H. J., PATEL, B., AND POONER, B. I. (1986). Increase in the number of type II insulin-like growth factor receptors during propylthiouracil-induced hyperplasia in the rat thyroid. *Endocrinology* 119, 1204-1209.
- POTTER, C. L., MOORE, R. W., INHORN, S. L., HAGEN, T. C., AND PETERSON, R. E. (1986). Thyroid status and thermogenesis in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 84, 45-55.
- POTTER, C. L., SIPES, I. G., AND RUSSEL, D. H. (1983). Hypothyroxinemia and hypothermia in rats in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administration. *Toxicol. Appl. Pharmacol.* 69, 89-95.
- PRESTON-MARTIN, S., BERNSTEIN, L., PIKE, M. C., MALDONADO, A. A., AND HENDERSON, B. E. (1987). Thyroid cancer in young women related to prior thy-

- roid disease and pregnancy history. *Brit. J. Cancer* 55, 191-195.
- PURVES, H. O. (1943). Studies on experimental goiter. IV. The effect of diiodotyrosine and thyroxine on the goitrogenic action of brassica seeds. *Brit. J. Exp. Pathol.* 24, 171-173.
- RICCABONA, G. (1982). Thyroid cancer and endemic goiter. In *Endemic Goiter and Endemic Cretinism* (J. B. Stanbury and B. S. Hetzel, Eds.), pp. 333-350. Wiley, NY.
- RICHTER, C. P., AND CLISBY, K. H. (1942). Toxic effects of the bitter-tasting phenylthiocarbamide. *Arch. Pathol.* 33, 46-57.
- ROBINSON, D. E., AND MITCHELL, A. D. (1981). Unscheduled DNA synthesis response in human fibroblasts, WI-38 cells, to 20 coded chemicals. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), p. 517-527. Elsevier, NY.
- ROGER, P. P., AND DUMONT, J. E. (1984). Factors controlling proliferation and differentiation of canine thyroid cells cultured in reduced serum conditions. Effects of L-thyrotropin, cyclic AMP, and growth factors. *Mol. Cell. Endocrinol.* 36, 79-93.
- ROGER, P. P., REUSE, S., SERVAIS, P., VAN HEUVERS-VYIN, B., AND DUMONT, J. E. (1986). Stimulation of cell proliferation and inhibition of differentiation expression by tumor-promoting phorbol esters in dog thyroid cells in primary culture. *Cancer Res.* 46, 898-906.
- ROJESKI, M. T., AND GHARIB, H. (1985). Nodular thyroid disease: Evaluation and management. *N. Engl. J. Med.* 313, 428-436.
- RON, E., KLEINERMAN, R. A., BOICE, J. D., LIVOLSI, V. A., FLANNERY, J. T., AND FRAUMENI, J. F. (1987). A population-based case-control study of thyroid cancer. *J. Natl. Cancer Inst.* 79, 1-12.
- RON, E., AND MODAN, B. (1982). Thyroid cancer. In *Cancer Epidemiology and Prevention* (D. Schottenfeld and J. F. Fraumeni, Eds.), pp. 837-854. Saunders, Philadelphia.
- ROOTWELT, K., GANES, T., AND JOHANNESSEN, S. I. (1978). Effect of carbamazepine, phenytoin and phenobarbitone on serum levels of thyroid hormones and thyrotropin in humans. *Scand. J. Clin. Lab. Invest.* 38, 731-736.
- ROSENKRANZ, H. S., HYMAN, J., AND LEIFER, Z. (1981). DNA polymerase deficient assay. In *Evaluation of Short Term Tests for Carcinogens* (J. de Serres and J. Ashby, Eds.), pp. 210-218. Elsevier, NY.
- ROSENKRANZ, H. S., AND POIRIER, L. A. (1979). Evaluation of the mutagenicity and DNA-modifying activity of carcinogenicity and noncarcinogenicity in microbial systems. *J. Natl. Cancer Inst.* 62, 873-892.
- ROSS, D. S., ELLIS, M. F., AND RIDGWAY, E. C. (1986). Acute thyroid hormone withdrawal rapidly increases the thyrotropin α - and β -subunit messenger ribonucleic acids in mouse thyrotropic tumors. *Endocrinology* 118, 1006-1010.
- ROZENGURT, E. (1986). Early signals in the mitogenic response. *Science* 234, 161-166.
- RYAN, D., LU, A. Y. H., AND LEVIN, W. (1978). Purification of cytochrome P-450 and P-448 from rat liver microsomes. *Methods Enzymol.* 52, 117-123.
- SABERI, M., STERLING, F. H., AND UTIGER, R. D. (1975). Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. *J. Clin. Invest.* 55, 218-223.
- SAR, M., TSUSHIMA, T., ISOZAKI, O., MURAKAMI, H., OHBA, Y., SATO, K., ARQI, M., MARIKO, A., AND SHIZUME, K. (1987). Interaction of insulin-like growth factor I with porcine thyroid cells cultured in monolayer. *Endocrinology* 121, 749-756.
- SALAMONE, M. F., HEDDLE, J. A., AND KATZ, M. (1981). Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 686-697. Elsevier, NY.
- SAMAAN, N. A., OSBORNE, B. M., MACKAY, B., LEAVENS, M. E., DUELLO, T. M., AND HALMI, N. S. (1977). Endocrine and morphologic studies of pituitary adenomas secondary to primary hypothyroidism. *J. Clin. Endocrinol. Metab.* 45, 903-911.
- SAMPSON, R. J., WOOLNER, L. B., BAHN, R. C., AND KURLAND, L. T. (1974). Occult thyroid carcinoma in Olmsted County, Minnesota: Prevalence at autopsy compared with that in Hiroshima and Nagasaki, Japan. *Cancer* 34, 2072-2076.
- SANTLER, J. E. (1957). Growth in the cell populations of the thyroid gland of rats treated with thiouracil. *J. Endocrinol.* 15, 151-161.
- SAP, J., MUNOZ, A., DAMM, K., GOLDBERG, Y., GHYSDAEL, J., LEUTZ, A., BEUG, H., AND VENNSTROMI, B. (1986). The c-erb-A protein is a high-affinity receptor for thyroid hormone. *Nature (London)* 324, 635-640.
- SCHAFFER, R., AND MULLER, H. A. (1980). On the development of metastasizing tumors of the thyroid gland after combined administration of nitrosomethylurea and methylthiouracil. *J. Cancer Res. Clin. Oncol.* 96, 281-285.
- SCHALLER, R. T., AND STEVENSON, J. K. (1966). Development of carcinoma of the thyroid in iodine deficient mice. *Cancer* 19, 1063-1080.
- SCHOTTENFELD, D., AND GERSHMAN, S. T. (1978). Epidemiology of thyroid cancer. *Ca Cancer J. Clin.* 28, 66-87.
- SCHWARTZ, H. L., BERNSTEIN, G., AND OPPENHEIMER, J. H. (1969). Effect of phenobarbital administration on the subcellular binding of 125 I-thyroxine in rat liver: Importance of microsomal binding. *Endocrinology* 84, 111-120.
- SCHUPBACH, M., AND HUMMLER, H. (1977). A comparative study on the mutagenicity of ethylenethiourea in bacterial and mammalian test systems. *Mutat. Res.* 56, 111-120.
- SEILER, J. P. (1974). Ethylenethiourea (ETU), a carcinogenic and mutagenic metabolite of ethylenedisithiocarbamate. *Mutat. Res.* 26, 189-191.
- SEILER, J. P. (1975). *In vivo* mutagenic interaction of nitrite and ethylenethiourea. *Experientia* 31, 214-215.
- SEILER, J. P. (1977). Nitrosation *in vitro* and *in vivo* by sodium nitrite, and mutagenicity of nitrogenous pesticides. *Mutat. Res.* 48, 225-236.
- SELJELID, R., HELMINEN, H. J., AND THIES, G. (1971). Effect of long-term suppression and stimulation of rat thyroid with special reference to lysosomes. *Exp. Cell Res.* 69, 249-255.
- SHAPIRO, S. J., FRIEDMAN, N. B., PERZIK, S. L., AND CATZ, B. (1970). Incidence of thyroid carcinoma in Graves' disease. *Cancer* 26, 1261-1270.
- SHARP, D. C., AND PARRY, M. (1981a). Induction of mitotic gene conversion by 41 coded compounds using the yeast culture JD1. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 491-501. Elsevier, NY.
- SHARP, D. C., AND PARRY, M. (1981b). Use of repair-deficient strains of yeast to assay the activity of 40 coded compounds. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 502-516. Elsevier, NY.
- SHIBASU, Y., MORIYA, M., KATO, K., LIENARD, F., WAZUKA, H., AND TERAMOTO, S. (1977). *Mutagenicity Screening on Pesticides and Modification Products: A Basis of Carcinogenicity*, Vol. 4, pp. 267-285. Cold Spring Harbor Conference on Cell Proliferation, Cold Spring Harbor Symposium, Cold Spring Harbor, NY.
- SHUPNIK, M. A., CHIN, W. W., HABENER, J. F., AND RIDGWAY, E. C. (1985). Transcriptional regulation of the thyrotropin subunit genes by thyroid hormone. *J. Biol. Chem.* 260, 2900-2903.
- SHUPNIK, M. A., ARDISON, L. J., MESKELL, M. J., BORNSTEIN, J., AND RIDGWAY, E. C. (1986). Triiodothyronine (T_3) regulation of thyrotropin subunit gene transcription is proportional to T_3 nuclear receptor occupancy. *Endocrinology* 118, 367-371.
- SILVERBERG, E., AND LUBERA, J. A. (1988). Cancer Statistics—1988. *Ca Cancer J. Clin.* 38, 5-22.
- SIMMON, V. F. (1979). *In vitro* assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J. Natl. Cancer Inst.* 62, 901-909.
- SHIMA, D., PASCAL, R., AND FURTH, J. (1965). Transplantable thyroid carcinoma induced by thyrotropin. *Arch. Pathol.* 79, 192-198.
- SLEIGHT, S. D., MANGKOEWDJOU, S., AKOSO, B. T., AND SANGER, V. L. (1978). Polybrominated biphenyl toxicosis in rats fed an iodine deficient, iodine adequate and iodine excess diet. *Environ. Health Perspect.* 23, 341-346.
- SMELAND, E., GODAL, T., RUUD, E., BEISKE, K., FANDELUND, S., CLARK, E. A., PFEIFER-OHLSSON, S., AND OHLSSON, R. (1985). The specific induction of myc protooncogene expression in normal human B cells is not a sufficient event for the acquisition of competence to proliferate. *Proc. Natl. Acad. Sci. USA* 82, 6255-6259.
- SMITH, D. M. (1984). Ethylenethiourea: Thyroid function in 2 groups of exposed workers. *Brit. J. Ind. Med.* 41, 362-366.
- SMITH, P., WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1986). Growth factor control of rat thyroid follicular cell proliferation. *Endocrinology* 119, 1439-1445.
- SMITH, P. J., AND SURKS, M. I. (1984). Multiple effects of 5,5'-diphenylhydantoin on the thyroid hormone system. *Endocr. Rev.* 5, 514-524.
- SRINIVASAN, V., MOUDGAL, M. R., AND SARMA, P. S. (1957). Studies on goitrogenic agents in food. I. Goitrogenic action of groundnut. *J. Nutr.* 61, 87-95.
- STANBURY, J. B., AIGINGER, P., AND HARBISON, M. D. (1979). Familial goiter and related disorders. In *Endocrinology* (L. J. De Groot et al., Eds.), Vol. 1, pp. 523-539. Grune and Stratton, NY.
- STANLEY, M. M., AND ASTWOOD, E. B. (1947). Determination of the relative activities of antithyroid compounds in man using radioactive iodine. *Endocrinology* 41, 66-84.
- STILES, C. D., CAPONE, G. T., SCHER, C. D., ANTONIADES, H. N., VAN WYK, J. J., AND PLEDGER, W. J. (1979). Dual control of cell growth by somatomedins and platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 76, 1279-1283.
- STRINGER, B. M. J., WYNFORD-THOMAS, D., AND WILLIAMS, E. D. (1985). *In vitro* evidence for an intracellular mechanism limiting the thyroid follicular cell growth response to thyrotropin. *Endocrinology* 116, 611-615.
- STUDZINSKI, G. P. (1988). Is c-myc protein directly involved in DNA replication? *Science* 240, 1202-1203.
- STUDZINSKI, G. P., BRELVI, Z. S., FELDMAN, S. C., AND WATT, R. A. (1986). Participation of c-myc protein in DNA synthesis in human cells. *Science* 234, 467-470.
- STYLES, J. A. (1980). Studies on the detection of carcinogens using a mammalian cell transformation assay with liver homogenate activation. In *Short-Term Test Systems for Detecting Carcinogens* (K. H. Norpoth and R. C. Garner, Eds.), pp. 226-238. Springer-Verlag, NY.
- STYLES, J. A. (1981). Activity of 42 coded compounds in the BHK 21 cell transformation test. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 638-646. Elsevier, NY.
- TANABE, A., NIELSEN, T., AND FIELD, J. B. (1984). Effects of TSH and phorbol ester on calcium-phospholipid dependent protein kinase of thyroid. *Clin. Res.* 32, 487A.
- TANAKA, K., INO, T., SAWAHATA, T., MARUI, S., IGAKI, H., AND YASHIMA, H. (1985). Mutagenicity of *N*-acetyl- and *N,N'*-diacetyl derivatives of 3 aromatic amines used as epoxyresin hardeners. *Mutat. Res.* 143, 11-15.
- TAUB, R., KIRSCH, I., MORTON, C., LENOIR, G., SWAN, D., TRONIK, S., AARONSON, S., AND LEDER, P. (1982). Translocation of the c-myc gene into the immunoglobulin heavy chain locus in Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl. Acad. Sci. USA* 79, 7837-7841.
- TAUROG, A. (1976). The mechanism of action of thio-

- ureylene antithyroid drugs. *Endocrinology* 98, 1031-1046.
- TAUROG, A. (1979). Hormone synthesis. In: *Endocrinology* (L. J. DeGroot, G. F. Cahill, Jr., L. Martini, D. H. Nelson, W. D. Odell, J. T. Potts, Jr., E. Steinberger, and A. I. Winegrad, Eds.), Vol. 1, pp. 331-342. Grune and Stratton, NY.
- TERAMOTO, S., SHINGU, A., AND SHIRASU, Y. (1978). Induction of dominant-lethal mutations after administration of ethylenethiourea in combination with nitrite or of N-nitrosoethylenethiourea in mice. *Mutat. Res.* 56, 335-340.
- TESTA, B., AND JENNER, P. (1976). *Drug Metabolism: Chemical and Biochemical Aspects*. Dekker, NY.
- THOMAS, J. A., AND BELL, J. U. (1982). Endocrine toxicology. In *Principles and Methods in Toxicology* (A. W. Hayes, Ed.), pp. 487-496. Raven Press, New York.
- THOMSON, J. A. (1981). Mutagenic activity of 42 coded compounds in the lambda induction assay. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 224-235. Elsevier, NY.
- TODD, G. C. (1986). Induction of reversibility of thyroid proliferative changes in rats given an antithyroid compound. *Vet. Pathol.* 23, 110-117.
- TOPHAM, J. C. (1980). Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? *Mutat. Res.* 74, 379-387.
- TRAMONTANO, D., CHIN, W. W., MOSES, A. C., AND INGBAR, S. H. (1986a). Thyrotropin and dibutylryl cyclic AMP increase levels of c-myc and c-fos mRNAs in cultured rat thyroid cells. *J. Biol. Chem.* 261, 3919-3922.
- TRAMONTANO, D., CUSING, G. W., MOSES, A. C., AND INGBAR, S. H. (1986b). Insulin-like growth factor-I stimulates the growth of rat thyroid cells in culture and synergizes the stimulation of DNA synthesis induced by TSH and Graves'-IgG. *Endocrinology* 119, 940-945.
- TRAMONTANO, D., MOSES, A. C., PICONE, R., AND INGBAR, S. H. (1987). Characterization and regulation of the receptor for insulin-like growth factor-I in the FRTL-5 rat thyroid follicular cell line. *Endocrinology* 120, 785-790.
- TRANSBOL, I., CHRISTIANSEN, C., AND BAASTRUP, P. C. (1978). Endocrine effects of lithium. *Acta Endocrinol.* 87, 759-767.
- Tsuchimoto, T., and Matter, B. E. (1981). Activity of coded compounds in the micronucleus test. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 705-711. Elsevier, NY.
- TSUDA, H., FUKUSHIMA, S., IMAIDA, K., KUNATA, Y., AND ITO, N. (1983). Organ-specific promoting effect of phenobarbital and saccharin in induction of thyroid, liver, and urinary bladder tumors in rats after initiation of N-nitrosomethylurea. *Cancer Res.* 43, 3292-3296.
- TSUTSUI, T., MAIZUMI, H., AND BARRETT, J. C. (1984). Amitrole-induced cell transformation and gene mutations in Syrian hamster embryo cells in culture. *Mutat. Res.* 140, 205-207.
- TU, A., HALLOWELL, W., PALLOTTA, S., SIVAK, A., LUBET, R. A., CURREN, R. D., AVERY, M. D., JONES, C., SEDITA, B. A., HUBERMAN, E., TENNANT, R., SPALDING, J., AND KOURI, R. E. (1986). An interlaboratory comparison of transformation in Syrian hamster embryo cells with model and coded chemicals. *Environ. Mutat.* 8, 77-98.
- TWEATS, D. J. (1981). Activity of 42 coded compounds in a differential killing test using *Escherichia coli* strains WP2, WP67 (uvr A pol A), and CM 871 (uvr A 1cx A rec A). In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 199-209. Elsevier, NY.
- U.S. EPA. (1986). Guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. *Fed. Regist.* 51, 33992-34003.
- U.S. EPA. (1988). *Reports of the EPA Gene-Tox Program*. Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.
- VALENCIA, R., AND HOUTCHENS, K. (1981). Mutagenic activity of 10 coded compounds in the *Drosophila* sex-linked recessive lethal test. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 650-659. Elsevier, NY.
- VALENTE, W. A., VITTI, P., ROTELLA, C. M., VAUGHAN, M. M., ALOJ, S. M., GOLLMAN, E. F., AMBESI-IMPOMBATO, F. S., AND KOHN, L. D. (1983). Antibodies that promote thyroid growth; A distinct population of thyroid-stimulating autoantibodies. *N. Engl. J. Med.* 309, 1028-1034.
- VANDERPAS, J., BOURDOUX, P., LAGASSE, R., et al. (1984). Endemic infantile hypothyroidism in a severe endemic goitre area of Central Africa. *Clin. Endocrinol.* 20, 327-340.
- VAN ETTEEN, C. H. (1969). Goitrogens. In *Toxic Constituents of Plant Foodstuffs* (I. E. Liener, Ed.), pp. 103-142. Academic Press, New York.
- VANSANDE, J., COCHAUX, P., MOCKEL, J., AND DUMONT, J. E. (1983). Stimulation by forskolin of the thyroid adenylate cyclase, cyclic AMP accumulation and iodine metabolism. *Mol. Cell. Endocrinol.* 29, 109-119.
- VENTURI, S., AND CROFTON-SLEIGH, C. (1981). Mutagenicity of 42 coded compounds in a bacteria assay using *Escherichia coli* and *Salmonella typhimurium*. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 351-360. Elsevier, NY.
- VICKERY, A. L. (1981). The diagnosis of malignancy in dysbornonogenetic goitre. *Clin. Endocrinol. Metab.* 10, 317-335.
- VOGEL, E., BLJLEVEN, W. G. H., KLAPWUK, P. M., AND ZIJLSTRA, J. A. (1980). Some current perspectives of the application of *Drosophila* in the evaluation of carcinogens. In *The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation* (G. M. Williams et al., Eds.), pp. 125-147. Elsevier/North-Holland, NY.
- VOGEL, E., BLJLEVEN, W. G. H., KORTSELIUS, M. J. H., AND ZIJLSTRA, J. A. (1981). Mutagenic activity of 17 coded compounds with sex-linked recessive lethal test in *Drosophila melanogaster*. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 660-665. Elsevier, NY.
- WAHNER, H. W., CUJELLO, C., COREA, P., URIBE, L. F., AND GAITAN, E. (1966). Thyroid carcinoma in an endemic goiter area, Cali, Colombia. *Amer. J. Med.* 40, 58-66.
- WATERHOUSE, J., MUIR, C., SHANMUGARTNAM, K., AND POWELL, J. (Eds.) (1982). *Cancer Incidence in Five Continents*, Vol. IV. IARC Publications No. 42, Lyon, France.
- WEGELIN, C. (1928). Malignant disease of the thyroid gland and its relationship to goitre in man and animals. *Cancer Rev.* 3, 297-313.
- WEINBERG, R. A. (1985). The action of oncogenes in the cytoplasm and nucleus. *Science* 230, 770-776.
- WEINBERGER, C., THOMPSON, C. C., ONG, E. S., LEBOR, R., GRUOL, J. J., AND EVANS, R. M. (1986). The c-erb-A gene encodes a thyroid hormone receptor. *Nature (London)* 324, 641-646.
- WEISBERGER, E. K., RUSSELD, A. B., HOMBERGER, F., WEISBERGER, J. H., BOGES, E., VANDONGEN, C. G., AND CHU, K. C. (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J. Environ. Pathol. Toxicol.* 2, 325-356.
- WEISBERGER, E. K., KRISHNA-MURTHY, A. S., LILJA, H. S., LAMB, J. C., IV. (1984). Neoplastic response of F344 rats and B6C3F1 mice to the polymer and dye-stuff intermediates 4,4'-methylenebis(N,N-dimethyl)benzenamine, 4,4'-oxydianiline and 4,4'-methylene-dianiline. *J. Natl. Cancer Inst.* 72, 1457-1463.
- WESTERMARK, K., KARLSSON, F. A., AND WESTERMARK, B. (1983). Epidermal growth factor modulates thyroid growth and function in culture. *Endocrinology* 112, 1680-1686.
- WESTERMARK, K., WESTERMARK, B., KARLSSON, F. A., AND ERICKSON, L. E. (1986). Location of epidermal growth factor receptors on porcine thyroid follicle cells and receptor regulation by thyrotropin. *Endocrinology* 118, 1040-1046.
- WILKINSON, C. F. (1984). Metabolism and selective toxicity in an environmental context. In *Foreign Compound Metabolism* (J. Caldwell and G. D. Paulson, Eds.), pp. 133-147. Taylor and Francis, London.
- WILLIAMS, E. D., DONIACH, I., BJARNASON, O., AND MICHIE, W. (1977). Thyroid cancer in an iodide rich area. *Cancer* 39, 215-222.
- WITTE, A., AND MCKENZIE, J. M. (1981). Regulation of the rat thyrotropin receptor *in vitro*. *Endocrinology* 108, 305-309.
- WOLLMAN, S. H., AND BREITMAN, T. R. (1970). Changes in DNA and weight of thyroid glands during hyperplasia and involution. *Endocrinology* 86, 322-327.
- WOO, Y.-T., LAI, D. Y., ARCOS, J. C., AND ARGOS, M. F. (1985). Chemical induction of cancer: Structural bases and biological mechanisms. In *Aliphatic and Polyhalogenated Carcinogens* (Y. Woo et al., Eds.), Vol. 3B, pp. 357-394. Academic Press, New York.
- WOODRUFF, R. C., MASON, J. M., VALENCIA, R., AND ZIMMERING, S. (1985). Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutat.* 7, 677-702.
- WOOLNER, L. B. (1969). Struma lymphomatosa and related disorders. *J. Clin. Endocrinol. Metab.* 19, 53-83.
- WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1982a). Goitrogen-induced thyroid growth in the rat: A quantitative morphometric study. *J. Endocrinol.* 94, 131-140.
- WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1982b). Dissociation of growth and function in the rat thyroid during prolonged goitrogen administration. *Acta Endocrinol.* 101, 210-216.
- WYNFORD-THOMAS, D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1982c). Desensitisation of rat thyroid to the growth-stimulating action of TSH during prolonged goitrogen administration. *Acta Endocrinol.* 101, 562-569.
- WYNFORD-THOMAS, K. D., STRINGER, B. M. J., AND WILLIAMS, E. D. (1986). Growth factor control of rat thyroid follicular cell proliferation. *Endocrinology* 119, 1439-1445.
- WYROBECK, A., GORDON, L., AND WATCHMAKER, G. (1981). Effect of 17 chemical agents including 6 carcinogen/noncarcinogen pairs on sperm shape abnormalities in mice. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 712-717. Elsevier, NY.
- YAMADA, T., AND LEWIS, A. E. (1968). An essential role of throxine and triiodothyronine balance in establishing normal pituitary-thyroid feedback control in goitrogen-treated rats. *Endocrinology* 82, 91-99.
- YAMADA, T., TSUKUI, T., IKEJIRI, K., YUKIMURA, Y., AND KOTANI, M. (1976). Volume of sella turcica in normal subjects and patients with primary hypothyroidism and hyperthyroidism. *J. Clin. Endocrinol. Metab.* 42, 817-822.
- YAMASHITA, S., ONG, J., FAGIRA, J. A., AND MEINED, S. (1986). Expression of the myc cellular protooncogene in human thyroid tissue. *J. Clin. Endocrinol. Metab.* 63, 1170-1173.
- YOSHIKURA, H., AND MATSUSHIMA, T. (1981). MLV test (integration enhancement test) of 42 coded compounds in mouse kidney cells. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 647-650. Elsevier, NY.
- ZAJAC-KAYE, M., GELMANN, E. P., AND LEVENS, D. (1988). A point mutation in the c-myc locus in Burkitt lymphoma abolishes binding of a nuclear protein. *Science* 240, 1776-1780.
- ZIMMERMANN, F. K., AND SCHEEL, I. (1981). Induction of mitotic gene conversion in strain D7 of *Saccharomyces cerevisiae* by 42 coded chemicals. In *Evaluation of Short Term Tests for Carcinogens* (F. J. de Serres and J. Ashby, Eds.), pp. 481-490. Elsevier, NY.

APPENDIX D

A REVIEW OF RECENT WORK ON THYROID REGULATION AND THYROID CARCINOGENESIS

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NOTICE

This report was prepared by Dr. Gordon C. Hard of the American Health Foundation initially under subcontract to Eastern Research Group, Inc. (ERG), Lexington, Massachusetts, for the U.S. Environmental Protection Agency (EPA) Risk Assessment Forum. ERG assembled and produced the final September 1992 report. Since then, the 1992 report has been further updated by Dr. Hard to include papers into 1996. The views presented are those of the author, and not necessarily those of EPA.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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INTRODUCTION

Most risk assessment issues involving the thyroid concern the role of the prolonged elevation of circulating thyroid-stimulating hormone (TSH) levels on the development of follicular cell neoplasia in laboratory animals and the appropriate procedures for extrapolation of these results to humans. A Technical Panel of the U.S. Environmental Protection Agency (EPA) Risk Assessment Forum (Forum) examined this issue and concluded in a 1988 draft report that, under certain circumstances, thyroid follicular cell tumors develop through an ordered linkage of steps beginning with interference in thyroid-pituitary status. When there is no direct interaction of the chemical with DNA, the Technical Panel concluded that thyroid follicular neoplasia involves a threshold process and would not develop unless there is prolonged interference with the thyroid-pituitary feedback mechanisms. The mechanistic information assembled in the 1988 draft was published in 1989 (Hill et al., 1989). The Forum is presently preparing a science policy statement for assessing risk of thyroid follicular cell neoplasia and requested an update of the pertinent literature as part of this process.

Since the EPA report on thyroid follicular cell carcinogenesis, was published (Hill et al., 1989), over 600 pertinent papers on thyroid function, regulation, carcinogenesis, and epidemiology have appeared in the literature. This review of the new publications highlights selected information on the mechanisms of normal and abnormal thyroid growth and function and the action of chemicals thereon.

Briefly, recent studies on regulation of the thyroid gland and thyroid follicular cell neoplasia present a broad array of new data that add depth and complexity to the information available in 1988 on this fundamental biological process. These studies provide information on growth factors and messenger systems, the control of TSH secretion in the central nervous system, the intrinsic heterogeneity in follicular cell populations with regard to proliferative potential, and new data on thyroid cancer in rodents and humans.

The studies also confirm previous suggestions regarding the importance of chemically induced thyroid peroxidase inhibition and the inhibition of 3,3',5,5'-tetraiodothyronine (T_4 , thyroxine) deiodinases on disruption of the thyroid-pituitary axis and thyroid neoplasia. In particular, new investigations that couple mechanistic studies with information from animal cancer bioassays (e.g., sulfamethazine studies) confirm the linkage between prolonged disruption of the thyroid-pituitary axis and thyroid neoplasia. Many new initiation/promotion studies also add to previous information suggesting that chronic stimulation of the thyroid induced by goitrogens can result in thyroid tumors.

It is now known that thyroid regulation occurs through a complex interactive network mediated through different messenger systems. Increased TSH levels activate the signal transduction pathways to stimulate growth and differentiation of the follicular cell. Although

oncogene activation and tumor suppressor gene inactivation may also be factors in the development of thyroid cancer, the important role of TSH on growth as well as function helps to explain how disruptions in the thyroid-pituitary axis may influence thyroid neoplasia.

Other new data from epidemiologic studies contribute to the understanding of thyroid neoplasia. Acute exposure to ionizing radiation, especially in childhood, remains the only verified cause of thyroid carcinogenesis in humans. Iodine deficiency studies as a whole remain inconclusive, even though several new studies in humans examine the role of dietary iodine deficiency in thyroid cancer.

EPA's analysis in the 1988 draft report focused on the use of a threshold for risk assessment of thyroid follicular tumors. New studies, involving several chemicals, provide further information that suggests there will be no antithyroid activity until critical intracellular concentrations are reached. Thus, for chemically induced thyroid neoplasia linked to disruptions in the thyroid-pituitary axis, a practical threshold for thyroid cancer would be expected. More information on thyroid autoregulation, the role of oncogene mutations and growth factors, and studies directly linking persistently high TSH levels with the sequential cellular development of thyroid follicular cell neoplasia would provide further confirmation.

THYROID REGULATION

Numerous recent studies point to the conclusion that the physiological regulation of thyroid cell growth and function involves a complex interactive network of trophic factors (endocrine, paracrine, and autocrine) and that the effects of these factors are mediated through a number of different second messenger systems. It is well established that TSH is the main growth factor for thyroid cells, maintaining as well the differentiated state of the thyroid and controlling thyroid hormone secretion. Other growth regulators involved in the complex web include insulin/insulinlike growth factor-I (IGF-I), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor β (TGF β), as well as an endogenous iodide-dependent mechanism (Wynford-Thomas, 1993; Farid et al., 1994).

TSH exerts its action on thyroid follicular cells via receptor sites, restricted mainly to the basal membranes of follicle cells (Mizukami et al., 1994). The advent of recombinant DNA technology has led to the cloning of the TSH receptor of both rat (Akamizu et al., 1990) and human thyroid (Libert et al., 1989; Nagayama et al., 1989; Parmentier et al., 1989; Misrahi et al., 1990). The TSH receptor is a plasma membrane site able to bind G (guanine nucleotide-binding) protein for signal transduction (Parmentier et al., 1989). G protein activation by the TSH receptor appears to be a highly complex effector system involving all four G protein families (Laugwitz et al., 1996). The gene for the TSH receptor is virtually constitutive in the thyroid cell, occurring far along the pathway of transformation, as demonstrated by persistent expression in

normal thyroid tissue as well as in differentiated thyroid tumors, but not undifferentiated carcinoma (Brabant et al., 1991). Current models indicate that the human TSH receptor is a heptahelical glycoprotein molecule with an extremely large extracellular domain at the N-terminus, a transmembrane/intracellular region consisting of seven intramembrane helices connected by three alternating intracellular and extracellular loops, and an intracellular tail at the carboxyl terminus (Nagayama and Rapoport, 1992; Vassart and Dumont, 1992). The extracellular domain is the ligand-binding site, conferring a high affinity for TSH binding and distinguishing it from other G protein-coupled receptors. It is thought that the three extracellular loops help the ligand to fit to the tertiary structure, while the intramembrane and intracellular segments appear to be critical for signal transduction. The available evidence indicates that the overall conformation of the TSH receptor in rats is probably the same as in humans.

TSH, through activation of its receptor, has been shown to stimulate multiple signal transduction pathways in the regulation of both growth and differentiated function. Each pathway may be related to specific cellular events. The main effector of TSH on proliferation and differentiation in a variety of species, man and rat included, is the cAMP signal transduction pathway, that is, the cascade involving activation of adenylate cyclase resulting in cAMP generation (Maenhaut et al., 1990; Dumont et al., 1992). There is increasing evidence that the physiological stimulation of thyroid cell function by TSH is achieved as well via the phosphatidylinositol/ Ca^{2+} (Pi-C) signal cascade, with activation of phospholipase C, in rat and human thyroid cells (Laurent et al., 1987; Leer et al., 1991; Dumont et al., 1992; D'Arcangelo et al., 1995), although, as a species difference, apparently not in dog thyroid cells (Mockel et al., 1991). Thus, the TSH receptor activates both pathways, but with a different efficacy, because in contrast to the cAMP pathway, much higher concentrations of TSH are required to stimulate the Pi-C phospholipase C cascade (Laurent et al., 1987). The signalling by these diverse pathways results in a range of metabolic consequences, including iodine uptake and release, thyroid peroxidase generation, organification of residues on thyroglobulin, thyroid hormone synthesis and release, and thyroid cell growth and division (Dumont et al., 1992). In this complex regulatory network, the TSH-cAMP cascade is functionally responsible for secretion, while the Pi-C phospholipase C cascade controls H_2O_2 generation and thyroid hormone synthesis (Corvilain et al., 1994). It is generally accepted that cAMP accounts for the mitogenic effect of TSH (Uyttersprot et al., 1995), although higher concentrations of TSH and more prolonged stimulation of the cAMP cascade are necessary to induce cell proliferation than for expression of differentiated function (Roger et al., 1988). cAMP also appears to play a central role in iodide uptake and metabolism by the follicular cell (Filetti and Rapoport, 1983, 1984) and in thyroglobulin and thyroid peroxidase (TPO) gene expression (Van Heuverswyn et al., 1985; Chazenbalk et al., 1987).

In addition to the above second messenger systems, the possibility of a nontranscriptional regulatory pathway involving a phosphorylation site on the TSH receptor that is kinase-C sensitive has also been suggested (Akamizu et al., 1990).

Growth factors also play a key role, along with TSH, in the complex regulation of thyroid cell proliferation. However, there are few data yet to explain how these trophic factors interact with the cell cycle to stimulate or inhibit cell division in the thyroid. There is evidence that both TSH receptor gene expression and thyroglobulin gene expression are under the control of insulin/IGF-1 as well as TSH, at a transcriptional level in the rat cell-line FRTL-5 (Santisteban et al., 1987; Takahashi et al., 1990; Saji et al., 1992). Similar evidence for a complex autoregulatory feedback mechanism involving insulin/IGF-1 operative at several levels of interactive signalling is accumulating for other primary thyroid cell culture systems (Gerard et al., 1989; Eggo et al., 1990). Collective data suggest that a complex interaction between the 1,2-diacylglycerol/protein kinase C (one of the bifurcating second messenger pathways of the Pi-C signal cascade) and the adenylyl cyclase signal transduction systems is important in the regulation of thyroid growth by TSH and IGF-1 (Fujimoto and Brenner-Gati, 1992). Thus, in rat and man, insulin/IGF-1 is considered a necessary cofactor for the action of TSH on follicle cells, synergizing with TSH to induce thyrocyte proliferation while maintaining differentiated function (Farid et al., 1994). In humans, it has been recorded that benign and malignant thyroid tumors produce increased levels of IGF-1 (Minuto et al., 1989; Williams et al., 1989). Such findings have led to the suggestion that emergent adenoma cells lose their dependence on exogenous IGF-1, acquiring the capability for autocrine production of this growth factor, resulting in continued autostimulation of cell replication and thus allowing thyroid nodules to become autonomous (Williams et al., 1988; Thomas and Williams, 1991).

Other autocrine/paracrine regulators of thyroid growth, with potent mitogenic activity for thyroid cells demonstrable in vitro include EGF and bFGF. EGF is synthesized within the thyroid gland and induces proliferation in thyroid cells from a wide range of species at the expense of dedifferentiation and loss of specialized thyroid-specific function (Asmis et al., 1995; Nilsson, 1995). bFGF is present in human thyroid tissue (Taylor et al., 1993), and there are stores of FGF in the basement membrane of follicles in adult normal rat thyroid (Logan et al., 1992). EGF has been shown to stimulate the growth and invasion of differentiated human thyroid cancer cells in culture and in nude mice (Hoelting et al., 1994), whereas bFGF expression increases during thyroid hyperplasia in the rat (Becks et al., 1994).

Transforming growth factor β_1 (TGF β_1) is a putative negative regulator of thyroid growth, as studies in all normal cell systems have shown it to inhibit thyrocyte proliferation, including that mediated by TSH (Morris et al., 1988; Colletta et al., 1989; Roger, 1996). Although the actual role of TGF β_1 in the thyroid is not known, cell culture and rodent studies suggest that it is a

limiting autocrine influence on thyroid cell hyperplasia and cancer growth (Hölting et al., 1994; Logan et al., 1994). In one study, TGF β_1 was detected in approximately 50% of human thyroid carcinomas, but not in adenomas, with a striking correlation being observed between the dual presence of TGF β_1 expression and arginine substitution at codon 61 of the *H-ras* oncogene (Jasani et al., 1990)

There also has been additional evidence that iodine is a major mediator of thyroid autoregulation, involving numerous inhibitory actions (Wolff, 1989). One of these is a decrease in cAMP formation in response to TSH, resulting in an inhibition of all cAMP-mediated stimulatory effects of TSH on the thyroid. Excess iodide therefore exerts a negative control on different thyroid parameters, inhibiting iodide uptake and organification, protein and RNA biosynthesis, hormone secretion, as well as paracrine mitogenic activity on endothelial cells and fibroblasts (Chazenbalk et al., 1988; Pisarev et al., 1988; Gärtner et al., 1990). Most of these actions appear to be mediated by an intracellular organified iodine intermediate of as yet unknown identity. Various derivatives of arachidonic acid have been proposed as this putative regulator(s), including the iodinated eicosanoid δ -iodolactone (Gärtner et al., 1990). However, this compound had no effect on TSH-mediated cAMP formation in porcine follicles (Dugrillon and Gärtner, 1995). Considered to be a more likely candidate is 2-iodohexadecanal (Boeynaems et al., 1995), a major iodolipid formed in horse thyroid when incubated with iodide (Pereira et al., 1990), but which is also detectable in the thyroid of other species, including man and rat.

METABOLISM AND EXCRETION OF THYROID HORMONES

There has been much recent progress in understanding the enzymatic pathways responsible for metabolism of T_4 , T_3 , and the inactive T_3 analog, reverse T_3 (rT_3). T_4 is secreted by the thyroid but has little biological activity unless deiodinated to T_3 . Two isoenzymes catalyze this 5'-deiodination reaction: type I 5'-deiodinase abundant in liver, kidney, and thyroid, and type II 5'-deiodinase found primarily in brain, pituitary, and brown adipose tissue (Leonard and Visser, 1986; Chanoine et al., 1993). In man, about 80% of circulating T_3 derives from peripheral 5'-monodeiodination of T_4 , particularly that by liver and kidney, while 20% of T_3 is secreted by the thyroid (Pekary et al., 1994). In the rat, intrathyroidal conversion of T_4 to T_3 provides the major source of T_3 (Chanoine et al., 1993), and rat thyroidal levels of the 5'-deiodinase are the highest so far reported for any species.

Recent studies have also confirmed that thyroid hormones in rats are metabolized predominantly through conjugation with either glucuronic acid or sulfate (de Herder et al., 1988; Eelkman Rooda et al., 1989; Visser et al., 1990). The enzymes responsible for glucuronidation of thyroid hormones are UDP-glucuronosyltransferases located mainly in the endoplasmic reticulum of the liver, but also of intestines and kidney. It appears that there are at least three UDP-GT

isoenzymes involved in rat liver. T_4 and rT_3 are glucuronidated by types I and II isoenzyme, while T_3 is glucuronidated by the type III isoform (Visser et al., 1993). The T_3 glucuronide conjugate is excreted in bile, which may represent a reversible pathway as the conjugate is hydrolyzed by intestinal bacteria, creating an enterohepatic cycle enabling reabsorption of free T_3 (de Herder et al., 1988; Rutgers et al., 1989a). The evidence also suggests that there may be a more effective enterohepatic circulation in humans than in rats (Rutgers et al., 1989a).

Sulfate conjugation of thyroid hormones is an alternative metabolic pathway. The sulfate conjugate of T_3 is rapidly deiodinated by type I deiodinase through successive deiodinations of the tyrosyl (inner) and phenolic (outer) rings (Visser et al., 1988), thus releasing iodine into the circulation for reutilization by the thyroid (Rutgers et al., 1989b). In man, the majority of nondeiodinative disposal of T_3 occurs via this pathway (LoPresti and Nicoloff, 1994).

CONTROL OF TSH SECRETION IN THE CENTRAL NERVOUS SYSTEM

At the central nervous system (CNS) level, recent work has provided additional information on the control of TSH secretion by thyroid hormones in the anterior pituitary and via the hypothalamus. A discrete population of neurons synthesizing thyrotropin-releasing hormone (TRH), located in the paraventricular nucleus of the hypothalamus, is under negative feedback regulation by circulating thyroid hormones (Koller et al., 1987; Segerson et al., 1987). Some results suggest that the biosynthesis of TRH is regulated by both T_3 and T_4 (Kakucska et al., 1992), although the mechanism by which T_4 plays an inhibitory role is unknown. The negative feedback of thyroid hormones on TSH secretion caused by antithyroid compounds appears to be exerted mainly at the pituitary level. This is because the increase in TRH release into hypophyseal portal blood produced by propylthiouracil (PTU) is relatively small (less than 50%) compared to the pronounced increase (up to 20 times at 3 weeks) in serum TSH (Rondeel et al., 1992). In rats, the data suggest that serum T_3 has a greater inhibitory action on TSH secretion from the pituitary than does serum T_4 (Emerson et al., 1989), at least in the euthyroid or mildly hypothyroid states. In humans, new highly sensitive immunometric assays used for measurement of TSH serum concentrations have underscored earlier work showing that thyroid hormone negative feedback on pituitary TSH secretion is mediated mainly by local generation of T_3 within the pituitary from T_4 by the 5'-deiodinase enzyme system (Spencer, 1996).

MITOGENIC EFFECT OF TSH ON THYROID TISSUE

Evidence concerning the mitogenic role of TSH for thyroid cells *in vivo* has been further consolidated over recent years. Studies from various laboratories employing tritiated thymidine labeling, metaphase-arrest techniques for mitotic index, or immunohistochemical decoration of statin (a nonproliferation-specific nuclear antigen identifying quiescent G₀-phase cells) show that

TSH stimulates, in a dose- and time-dependent way, the recruitment of noncycling G₀ cells into the cycling compartment, entry into S-phase, and entry of G₂ cells into mitosis (Bayer et al., 1992).

Recent work has indicated that the normal rodent (and human) thyroid may have an intrinsic heterogeneity in the follicular cell population regarding the capacity for proliferative response to TSH. One hypothesis suggests that there are a few subsets of stemlike follicular cells having a high growth potential compared to the majority of the population, and that this trait is stable and heritable (Peter et al., 1985; Smeds et al., 1987; Groch and Clifton, 1992; Studer and Derwahl, 1995). According to this model, the clones of cells with extensive proliferative potential are the origin of the adenomas that develop under conditions of chronic TSH stimulation (Smeds et al., 1987; Groch and Clifton, 1992).

As suggested by Studer et al. (1989), the intrinsic stem cell clone model reconciles earlier kinetic observations (Wynford-Thomas et al., 1982; Christov, 1985) that hyperstimulated thyroid attains a plateau phase of growth or state of "desensitization," with the vast majority of follicular cells becoming refractory to the mitogenic effects of increased TSH levels, before the emergence of adenomas. In this model, chronic TSH stimulation would select preexisting thyrocyte clones with the greatest proliferative potential, and thus greater risk of neoplastic transformation. An alternative model for explaining the self-limited nature of hypothyroidism and the development of hormone-responsive tumors in the chronically stimulated rat thyroid proposes that clones of cells escape from the desensitization mechanism through mutational events and natural selection, leading ultimately to tumor formation (Thomas and Williams, 1991). Both models agree that thyroid carcinogenesis involves rare subsets of cells responsive to continued TSH stimulation but differ concerning the origin of follicular cell heterogeneity. Additional support for the intrinsic subset concept and/or the controlling influence of TSH on the development of selected clones of thyroid follicular cells comes from other studies on cell proliferation (Bayer et al., 1992), or those using transplantation methodology (Watanabe et al., 1988; Domann et al., 1990; Ossendorp et al., 1990).

RODENT THYROID CANCER STUDIES

Although additional bioassays have revealed new compounds with thyroid tumor-inducing capability in rodents, such as malonaldehyde (NTP, 1988), or have provided stronger evidence of tumorigenicity as in the case with sulfamethazine (Littlefield et al., 1989, 1990), the most significant studies in this area concern the promoting activity of antithyroid compounds. These studies have employed N-bis(2-hydroxypropyl)nitrosamine (DHPN) as the initiating agent. Promoting activity in the rat has been confirmed for many antithyroid compounds, including thiourea and potassium thiocyanate (Kanno et al., 1990), PTU and potassium perchlorate (Hiasa

et al., 1987), 4,4'-methylenebis'(N,N-dimethyl)-benzenamine (MDBA) (Kitahori et al., 1988), 2,4-diaminoanisole sulfate (Kitahori et al., 1989), sulfadimethoxine (Mitsumori et al., 1995), and phenobarbital (McClain et al., 1988; Kanno et al., 1990). Where thyroid gland and hormone parameters were measured, there were strong correlations between the tumor-promoting activity on the one hand and increased thyroid weight, decreased serum T₃ and T₄ levels, and increased circulating TSH on the other (Kitahori et al., 1988, 1989; McClain et al., 1988). One of these studies (with MDBA) also linked the increased circulating level of TSH with increased numbers of TSH-positive cells in the anterior lobe of the pituitary gland (Kitahori et al., 1988). The phenobarbital study was noteworthy in demonstrating a clear dose-dependent negation of thyroid tumor promotion and plasma TSH elevation by T₄ replacement therapy (McClain et al., 1988). In the investigations where cell proliferative activity has been determined, the results strongly support an important role for high serum levels of TSH in the early stages of thyroid tumorigenesis (Shimo et al., 1994; Mitsumori et al., 1995).

These tumor promotion studies add further and consistent support to the hypothesis that antithyroid compounds exert effects secondarily on the thyroid through the chronic stimulation of persistently elevated levels of TSH.

EFFECTS OF SPECIFIC CHEMICALS ON THE THYROID-PITUITARY AXIS

Several recent studies have provided more quantitative data on the time- and dose-dependent effects of specific antithyroid chemicals on thyroid hormone status, including PTU (de Sandro et al., 1991), sulfamethazine (McClain, 1995), and phenobarbital (McClain et al., 1988; de Sandro et al., 1991). These studies defined the effects as early but persistent decreases in circulating T₃ and T₄ levels and a substantial increase in circulating TSH. Particularly noteworthy are observations on sulfamethazine where the dose-responsiveness for thyroid parameters in rats was studied with 10 dose levels spanning 3 orders of magnitude (McClain, 1995). Consistently, the parameters of thyroid weight and plasma T₃, T₄, and TSH levels displayed nonlinear dose-response curves with a major break in linearity from zero slope at around the 1,600 ppm dose level. These data suggested that, if coincident with tumor incidence data, a benchmark approach might conceivably be applied through the most sensitive indicator to provide a scientific basis for high- to low-dose extrapolation for secondary thyroid carcinogenesis.

There is also more detailed information available on the metabolic pathways and metabolites of such antithyroid compounds as methylmercaptoimidazole (MMI) and PTU (Taurog and Dorris, 1988; Taurog et al., 1989). Some of these data confirm the importance of antithyroid drugs accumulating in the target organ and thus achieving effective intracellular concentrations at the intrathyroidal site of iodide organification as a requirement for antithyroid activity. Accordingly, it has been calculated that the intracellular concentration of PTU in the rat thyroid

reaches approximately 20 μM (Taurog and Dorris, 1989), sufficiently high for iodination inhibition.

A particularly critical site of action representing a common intrathyroidal mechanism shared by PTU, ethylenethiourea (ETU), MMI, and aminotriazole (ATZ) is TPO inhibition. Antithyroid chemicals appear to be able to inactivate TPO in one of two ways, either by a reversible reaction that does not involve covalent binding, or by an irreversible interaction involving suicide inactivation of the enzyme. The latter reaction comprises branched pathways that proceed concurrently, namely, inactivation of the enzyme and turnover of the suicide substrate (Doerge, 1988). Thus, suicide inhibitors inactivate TPO by covalent binding to the prosthetic heme group in the presence of H_2O_2 , resulting in H_2O_2 -dependent catalytic formation of reactive intermediates (Doerge, 1988; Doerge and Niemczura, 1989). In this case, de novo synthesis is required to restore the lost enzyme activity (Doerge and Takazawa, 1990). The reaction between either PTU or ETU and the TPO/ H_2O_2 generating system is reversible, representing metabolic detoxification of these compounds in thyroid and not suicide inactivation (Doerge, 1988; Taurog and Dorris, 1989; Doerge and Takazawa, 1990). This contrasts with the effects of MMI and other thiocarbamide goitrogens, as well as ATZ, which cause suicide inactivation of the enzyme via covalent binding (Doerge, 1988; Doerge and Niemczura, 1989; Doerge and Takazawa, 1990). The difference in action between PTU or ETU and MMI, involving reversible inhibition of TPO on the one hand, and on the other, irreversible inactivation requiring de novo synthesis of enzyme to restore activity, may account for the longer duration of effect and greater clinical potency of MMI (Doerge and Takazawa, 1990). The different mechanism of TPO inactivation between compounds would not necessarily implicate a different overall mechanism of rodent thyroid carcinogenesis. However, the commonality of this intrathyroidal mechanism among antithyroid chemicals that appear to induce follicular cell carcinogenesis via secondary effects on the thyroid/pituitary axis suggests that TPO inactivation might be a useful additional criterion for categorizing these chemicals. It has been suggested further, that for risk assessment purposes, the effect on TPO provides a biochemical basis for the existence of a no-observed-effect level (NOEL) in chemically induced thyroid toxicity (Doerge and Takazawa, 1990).

A marked species difference may exist between primates and rodents in the inhibition of TPO by antithyroid compounds. As examples, inhibition of monkey TPO requires approximately 50 and 450 times the concentration of PTU and sulfamonomethoxine, respectively, than does rat TPO (Takayama et al., 1986). This inequality might explain the greater susceptibility of the rat to the antithyroid effects of such compounds compared to the primate.

More recent data concerning the extrathyroidal action of antithyroid compounds on the peripheral conversion of T_4 and T_3 involving inhibition of T_4 deiodinases primarily in liver

(reviewed by Curran and De Groot, 1991) is available for PTU (de Sandro et al., 1991), ATZ (Cartier et al., 1985), and phenobarbital (McClain et al., 1989; de Sandro et al., 1991; Barter and Klaassen, 1992). Unlike PTU, ATZ did not affect the outer ring 5'-deiodination pathway but stimulated inner ring 5-deiodination of T_4 with a consequent increase in serum concentration of rT_3 (Cartier et al., 1985), an inactive form of T_3 . ATZ has no thiocarbonyl (or aromatic) group in its structure, and the lack of sulfur could be a possible explanation for the difference in peripheral action from PTU. The studies with phenobarbital have confirmed that this drug acts through an extrathyroidal mechanism by increasing both hepatic glucuronidation of T_4 as well as increasing the clearance of T_4 from serum (McClain et al., 1989; de Sandro et al., 1991; Barter and Klaassen, 1992). These effects were mediated by the substantial induction of the enzyme responsible for T_4 metabolism, as well as an increased biliary flow. The effect of phenobarbital solely on the peripheral pathway and the less potent consequence for thyroid hormone levels, compared to the effects of a thiourylene compound like PTU, accords with its role as a promoter rather than an inducer of rodent thyroid tumorigenesis. There is still no epidemiologic evidence that chemicals such as phenobarbital, which affect thyroid function through a peripheral mechanism involving thyroid hormone metabolism, are associated with thyroid neoplasia in humans (McClain, 1989; Curran and De Groot, 1991).

In keeping with an indirect action on TSH hypersecretion from the anterior pituitary, some antithyroid compounds have been shown to have CNS effects. Qualitative increases in TSH-producing thyrotrophs in the rat pituitary have been associated with administration of PTU (Samuels et al., 1989) and 4,4'-oxydianiline (Murthy et al., 1985), with a rapid reversal of morphological changes upon cessation of treatment in the case of PTU. In the PTU study, there was also a coincident decline in growth hormone cells, suggesting transdifferentiation of somatotrophs into thyrotrophs (Horvath et al., 1990), thus implying changes in cell type rather than total cell number in the hypothyroid state. At the molecular level, this accords with the induction of increased levels of TSH mRNA in the anterior pituitary by PTU and a concomitant fall in growth hormone mRNA (Franklyn et al., 1986; Wood et al., 1987). The profound effect on cytoplasmic TSH levels affected both TSH β and α subunits (Franklyn et al., 1986; Mirell et al., 1987; Samuels et al., 1989), but the increases were relatively greater in TSH β than in the α subunit. T_3 replacement reversed these specific subunit changes (Samuels et al., 1989). These effects of PTU were dose and time dependent, and the various studies confirmed a direct influence of "thyroid status" on the regulation of pituitary hormones at a pretranslational level.

Importantly, there is evidence that genotoxic chemicals able to induce thyroid cancer in rodents have different morphological and physiological effects from those of known goitrogens. Thus, DHPN induces rat thyroid tumors along a multistage pathway involving focal atypical hyperplasia (originating from single follicles) rather than diffuse follicular hyperplasia (Kawaoui et

al., 1991). Furthermore, DHPN and N-nitrosomethylurea (NMU) appear not to influence the thyroid-pituitary axis during the induction of thyroid carcinogenesis because these compounds did not increase thyroid weights unrelated to tumor development or cause a persistent elevation of serum TSH levels or changes in serum T₄ levels (Mori et al., 1990; Hiasa et al., 1991; Hiasa et al., 1992).

Collectively, these studies with a range of compounds strengthen the hypothesis that antithyroid agents in rodents act by secondarily causing sustained elevations in serum TSH levels associated with the development of thyroid carcinogenesis. They also highlight the differences in pertinent effects between antithyroid compounds and those rodent thyroid carcinogens that are directly DNA reactive.

EPIDEMIOLOGY AND ETIOLOGY OF HUMAN THYROID CANCER

In man, tumor histology is important to the understanding of the etiology of thyroid cancer because different types appear to represent separate biological entities with different clinical and epidemiologic features (Pettersson et al., 1991, 1996). The most frequent type is papillary carcinoma, accounting for approximately 60% of all thyroid cancers, while follicular carcinoma represents about 20% (Goepfert and Callender, 1994). In Sweden, there are regional differences in the incidence of papillary and follicular types of thyroid cancer defined by iodine status, iodine-deficient areas being associated with a higher risk of follicular cancer (Pettersson et al., 1996). There is also some evidence that the incidence rates of these histologic entities may be changing. The data from one study reflect an increase for papillary thyroid cancer in Sweden since 1919 but a decline for follicular cancer in cohorts born since 1939 (Pettersson et al., 1991). Although residence in endemic goiter areas in Switzerland was linked to a modestly increased probability of developing thyroid cancer (Levi et al., 1991), overall, there remains a general view that no convincing evidence has yet emerged to link environmental thyroid cancer with areas of iodine deficiency. Furthermore, the long-standing program of supplementation of food items with iodine in Sweden has not affected thyroid cancer trends in iodine-deficient or iodine-rich areas (Pettersson et al., 1996). Vegetables known to contain, or endogenously generate, thiocyanate have not been found to enhance the risk of thyroid cancer, but possibly exert a protective influence (Franceschi et al., 1993). A meta-analysis of four similarly designed case-control studies conducted in high-thyroid cancer areas of Switzerland and Italy revealed an association with diets rich in starchy foods and fats, while raw ham and fish were protective (Franceschi et al., 1991).

The only verified cause of thyroid cancer in humans is exposure to ionizing radiation. This association has been established for x-radiation therapy (de Vathaire et al., 1988; Hawkins and Kingston, 1988; Ron et al., 1989; Tucker et al., 1991) and for radioactive fallout (Kerber et al., 1993; Nikiforov et al., 1996). Of the several events exemplifying the latter, the Chernobyl nuclear

power-plant disaster provides the most striking correlation. Since 1990, a very high incidence of childhood thyroid cancer has been recorded in the Republic of Belarus, affecting predominantly children who were less than 1 year old at the time of the accident (Nikiforov et al., 1996). Almost all of the cases have been papillary carcinomas with short latency (Nikiforov and Gnepp, 1994), in keeping with the observation that radiation-associated thyroid tumors are predominantly of the papillary type (Goepfert and Callender, 1994). The most biologically significant isotopes released in the fallout were radioiodines, primarily ^{131}I , and consequently radioiodine has been accepted as the causative factor (Williams, 1996). This stands in marked contrast to the lack of evidence incriminating diagnostic or therapeutic doses of ^{131}I (Holm et al., 1988, 1991). As with the Chernobyl experience, age at the time of treatment with x-radiation therapy is also an important factor in thyroid cancer development, the 67-fold risk at 12 years mean age declining to nonsignificance at a mean age of 29 years (Tucker et al., 1988). In contrast to the risk posed by high-level ionizing radiation, a well-designed Chinese study indicates that lifetime exposure to low-level environmental radiation, with an estimated cumulative dose of 9cGy, is not a risk factor for human thyroid cancer (Wang et al., 1990).

Graves' disease, an autoimmune thyroid condition, is associated with the presence of circulating antibodies stimulating the TSH receptor (TSAb) (Rees Smith et al., 1988; Paschke et al., 1995). Whether this disease carries an increased risk of thyroid carcinoma has been controversial. Nevertheless, from the collective published reports, Mazzaferri (1990) concludes that thyroid cancer incidence in surgically treated Graves' disease patients is between 5% and 10%. It is generally agreed that thyroid cancer occurring in Graves' disease is an aggressive form (Belfiore et al., 1990). There is strong support for a pathophysiological role of TSAb rather than circulating TSH in thyroid cancer development associated with Graves' disease (Filetti et al., 1988; Belfiore et al., 1990), particularly as serum TSH levels are suppressed in hyperthyroid patients with thyroid cancer, while TSAb's are present in most cases (Belfiore et al., 1990). Evidence that TSAb's are circulating autoantibodies to the TSH receptor comes from the finding that they, and monoclonal antibodies to the human TSH receptor in thyroid tissue, mimic many of the activities of TSH on thyroid cells (Rees Smith et al., 1988; Belfiore et al., 1990; Marion et al., 1992). The role of TSH in mediating the growth of thyroid nodules in humans needs further clarification, however (Ridgway, 1992).

CHANGES IN GENE EXPRESSION IN THYROID CARCINOGENESIS

To date, there have been numerous studies at the molecular level exploring the changes in gene expression that might accompany human thyroid carcinogenesis, involving a wide range of protooncogenes, oncogenes, tumor suppressor genes, or gene protein products. Despite the

technical evolution from transfection methodology to polymerase chain reaction amplification coupled with sequence-specific oligonucleotide hybridization or probing, some of the findings are discordant. For example, conflicting results have been obtained for the *erb* family of “nuclear” oncogenes, and for involvement of mutations of the TSH receptor or retinoblastoma (Rb) gene in thyroid tumor development, while other investigations have indicated clear evidence of no role in thyroid cancer for mutation of the APC, p16^{INK4} or nm23 genes, or for abnormal expression of the central regulating genes, *myc*, *myb*, *fos*, or *jun* (Wynford-Thomas, 1993; Fagin, 1994; Farid et al., 1994; Said et al., 1994).

There is general agreement emerging that some of the genetic changes observed can be correlated with tumor histotype and stage of tumor development. Point mutations of *ras* are the most frequent single genetic abnormalities found in human thyroid tumors, occurring in about 50% of those of follicular cell type (Wynford-Thomas, 1993; Fagin, 1994; Said et al., 1994), and these mutations are regarded as early molecular events in the development of this tumor type (Lemoine et al., 1989; Wright et al., 1989; Shi et al., 1991). Although all three *ras* family members have been involved (that is, Ha-*ras*, Ki-*ras*, N-*ras*), the changes have been associated mainly with Ha-*ras* mutation, the most common mutation site being codon 61 with glutamine>arginine substitution (Wright et al., 1989; Namba et al., 1990; Shi et al., 1991). When comparing the data for thyroid cancer associated with areas of iodine sufficiency, one study found a higher rate of *ras* mutation in thyroid tumors from iodine-deficient areas (Shi et al., 1991). Also prevalent in follicular adenomas are *gsp* mutations, occurring in about 25% of cases with a possible predilection for microfollicular adenomas (Suarez et al., 1991; O'Sullivan et al., 1991; Farid et al., 1994; Said et al., 1994). This distribution suggests that *gsp* mutation is another early event in the development of follicular thyroid cancer, although there appear to be some discrepancies between studies concerning the involvement of G proteins in thyroid neoplasia (Esapa et al., 1997).

In contrast to follicular tumors, rearrangements of *ret* and *trk* protooncogenes are associated with the papillary type of cancer. The *ret*/PTC rearrangement is specific for the thyroid and found in up to 30% of human papillary carcinomas (Jhiang and Mazzaferri, 1994; Said et al., 1994; Viglietto et al., 1995). As this alteration has been detected in over 40% of occult papillary carcinomas, generally considered to be the early stage of papillary malignancy, it is believed to represent an early event in the development of this tumor histotype (Viglietto et al., 1995). A higher prevalence of *ret* protooncogene rearrangement, in up to two-thirds of cases, has now been recorded in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear reactor accident (Fugazzola et al., 1995; Klugbauer et al., 1995). Unlike the situation in adult tumors where the most common *ret* translocation is *ret*/PTC1, the alteration in the childhood radiation tumors from Belarus was preferentially *ret*/PTC3 rearrangement (Fugazzola et al., 1995;

Klugbauer et al., 1995). Interestingly, the only reproducible cytogenetic abnormality found in papillary thyroid cancer has been an inversion of chromosome 10 at the 10q11.2 locus, which is known to involve the *ret* protooncogene at that locus (Donghi et al., 1989; Jenkins et al., 1990). Activating rearrangement of the *trk* protooncogene has also been found only in papillary carcinomas (Fagin, 1994; Said et al., 1994), while overexpression of the *met* oncogene is another molecular aberration observed mainly in papillary thyroid cancer (Said et al., 1994; Farid et al., 1995).

Little is known concerning molecular changes involved in the transition from adenoma to carcinoma, although in follicular tumors a loss of heterozygosity involving chromosome 3p was considered to be specific for follicular carcinoma, appearing to correlate with the transition from the adenoma to the carcinoma stage (Herrmann et al., 1991). Chromosomal analysis of follicular thyroid tumors has also indicated the existence of three cytogenetically distinct subsets of adenoma, with numerical changes in chromosomes 5, 7, and 12 as the most frequent cluster of anomalies (Roque et al., 1993a). A similar cluster of alterations found in some thyroid nodular hyperplasias has been interpreted as support for a biological continuum between hyperplastic nodules and the most common subset of adenomas (Roque et al., 1993b). At the histologic level, polysomies for chromosomes 7 and/or 12 have been observed only in lesions with an exclusive or predominant microfollicular component (Criado et al., 1995). There is some evidence from several studies that mutation of the tumor suppressor gene p53 is a late genetic event in thyroid carcinogenesis involved in the progression to a more aggressive phenotype in the form of undifferentiated or anaplastic cancer (Nakamura et al., 1992; Ito et al., 1993; Nikiforov et al., 1996).

Genetic alterations have also been detected in rat thyroid carcinogenesis, but only a few investigations have been reported. *Ha-ras* activation was exclusively involved in a majority of tumors induced by the direct-acting genotoxin NMU (Lemoine et al., 1988). In DHPN-induced rat thyroid tumors, however, mutations involved the *Ki-ras* gene via a G to A transition at the second base of codon 12 (Kitahori et al., 1995). The same point mutation was detected at an early time point in preneoplastic thyroid, suggesting that *Ki-ras* mutations may play a role in the development of DHPN-induced rodent thyroid cancer. In about half of the cases, radiation-induced thyroid tumors in rats were associated preferentially with *Ki-ras* activation (Lemoine et al., 1988). In this respect the rat data conform with that for radiation-induced papillary thyroid tumors in humans, which also are associated with *Ki-ras* mutation (Wright et al., 1991). On the other hand, ATZ-induced adenomas in the rat showed only a very low incidence of *Ki-ras* activation (Lemoine et al., 1988).

DATA GAPS AND RESEARCH NEEDS

Much of the recent new data strengthen the hypothesis that nongenotoxic antithyroid compounds induce rodent thyroid follicular cell carcinogenesis by an indirect mechanism involving thyroid-pituitary feedback regulation, and none of the data negate that notion. Some of the new evidence also supports the view that humans may be less sensitive to the antithyroid process than rodents. Nevertheless, there are gaps in the available information that require further research.

1. Despite the voluminous literature on thyroid autoregulation, more research is needed before the complex interactive network is fully elucidated.
2. More data are required to clarify the role and interaction of oncogene mutations and growth factor alterations in thyroid carcinogenesis in both rodents and humans, and particularly in defining differences, if any, between rodent thyroid cancer induced by antithyroid compounds compared to the action of genotoxic carcinogens.
3. More information is required concerning the differences that might distinguish the thyroid-related mechanisms of action of antithyroid compounds versus genotoxic carcinogens, such as DNA adduct analysis within the thyroid, and the effects of DNA-reactive carcinogens on TPO.
4. More information is required directly linking persistently high levels of circulating TSH with a step-by-step sequence involved in the cellular development of thyroid follicular cell neoplasia.
5. Well-conducted studies are needed to better define the comparative sensitivity of humans, relative to rodents, to the long-term effects of antithyroid factors.

REFERENCES

- Akamizu T, Ikuyama S, Saji M, Kosugi S, Kozac C, McBride OW, Kohn LD. (1990) Cloning, chromosomal assignment, and regulation of the rat thyrotropin receptor: expression of the gene is regulated by thyrotropin, agents that increase cAMP levels, and thyroid autoantibodies. *Proc. Natl. Acad. Sci.* 87:5677-5681.
- Asmis LM, Gerber H, Kaempf J, Studer H. (1995) Epidermal growth factor stimulates cell proliferation and inhibits iodide uptake of FRTL-5 cells *in vitro*. *J. Endocrinol.* 145:513-520.
- Barter RA, Klaassen CD. (1992) UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extra-thyroidal mechanism. *Toxicol. Appl. Pharmacol.* 113:36-42.

Bayer I, Mitmaker B, Gordon PH, Wang E. (1992) Modulation of nuclear statin expression in rat thyroid follicle cell following administration of thyroid stimulating hormone. *J. Cell. Physiol.* 150;276-282.

Becks GP, Logan A, Phillips ID, Wang J-F, Smith C, DeSousa D, Hill DJ. (1994) Increase of basic fibroblast growth factor (FGF) and FGF receptor messenger RNA during rat thyroid hyperplasia: temporal changes and cellular distribution. *J. Endocrinol.* 142;325-338.

Belfiore A, Garofalo MR, Giuffrida D, Runello F, Filetti S, Fiumara A, Ippolito O, Vigneri R. (1990) Increased aggressiveness of thyroid cancer in patients with Graves' disease. *J. Clin. Endocrinol. Metab.* 70;830-835.

Boeynaems J-M, van Sande J, Dumont JE. (1995) Which iodolipids are involved in thyroid autoregulation: iodolactones or iodoaldehydes? *Eur. J. Endocrinol.* 132;733-734.

Brabant G, Maenhaut C, Köhrle J, Scheumann G, Dralle H, Hoang-Vu C, Hesch RD, von zur Mühlen A, Vassart G, Dumont JE. (1991) Human thyrotropin receptor gene: expression in thyroid tumors and correlation to markers of thyroid differentiation and dedifferentiation. *Molec. Cell. Endocrinol.* 82;R7-R12.

Cartier L-J, Williams IK, Holloszy J, Premachandra BN. (1985) Potentiation of thyroxine 5-deiodination by aminotriazole. *Biochim. Biophys. Acta* 843;68-72.

Chanoine J-P, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S, Leonard JL. (1993) The thyroid gland is a major source of T₃ in the rat. *J. Clin. Invest.* 91;2709-2713.

Chazenbalk G, Magnusson RP, Rapoport B. (1987) Thyrotropin stimulation of cultured thyroid cells increases steady state levels of the messenger ribonucleic acid for thyroid peroxidase. *Molec. Endocrinol.* 1;913-917.

Chazenbalk GD, Valsecchi RM, Krawiec L, Burton G, Juvenal GJ, Monteagudo E, Chester HA, Pisarev MA. (1988) Thyroid autoregulation. Inhibitory effects of iodinated derivatives of arachidonic acid on iodine metabolism. *Prostaglandins* 36;163-172.

Christov K. (1985) Cell population kinetics and DNA content during thyroid carcinogenesis. *Cell Tissue Kinet.* 18;119-131.

Colletta G, Cirafici AM, DiCarlo A. (1989) Dual effect of transforming growth factor β on rat thyroid cells: inhibition of thyrotropin-induced proliferation and reduction of thyroid-specific differentiation markers. *Cancer Res.* 49;3457-3462.

Corvilain B, Laurent E, Lecomte M, Van Sande J, Dumont JE. (1994) Role of the cyclic adenosine 3',5'-monophosphate and the phosphatidylinositol-Ca²⁺ cascades in mediating the effects of thyrotropin and iodide on hormone synthesis and secretion in human thyroid slices. *J. Clin. Endocrinol. Metab.* 79;152-159.

Criado B, Barros A, Suijkerbuijk RF, Olde Weghuis D, Seruca R, Fonseca E, Castedo S. (1995) Detection of numerical alterations for chromosomes 7 and 12 in benign thyroid lesions by *in situ* hybridization. Histological implications. *Am. J. Pathol.* 147;136-144.

Curran PG, De Groot LJ. (1991) The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Rev.* 12;135-150.

D'Arcangelo D, Silletta MG, Di Francesco AL, Bonfitto N, Di Cerbo A, Falasca M, Corda D. (1995) Physiological concentrations of thyrotropin increase cytosolic calcium levels in primary cultures of human thyroid cells. *J. Clin. Endocrinol. Metab.* 80;1136-1143.

de Herder WW, Bonthuis F, Rutgers M, Otten MH, Hazenberg MP, Visser TJ. (1988) Effects of inhibition of type I iodothyronine deiodinase and phenol sulfotransferase on the biliary clearance of triiodothyronine in rats. *Endocrinology* 122;153-157.

de Sandro V, Chevrier M, Boddaert A, Melcion C, Cordier A, Richert L. (1991) Comparison of the effects of propylthiouracil, amiodarone, diphenylhydantoin, phenobarbital, and 3-methylcholanthrene on hepatic and renal T₄ metabolism and thyroid gland function in rats. *Toxicol. Appl. Pharmacol.* 111;263-278.

de Vathaire F, Francois P, Schweisguth O, Oberlin O, Le MG. (1988) Irradiated neuroblastoma in childhood as potential risk factor for subsequent thyroid tumour. *Lancet* 2;455.

Doerge DR. (1988) Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogens. Identification of turnover and inactivation pathways. *Biochemistry* 27;3697-3700.

Doerge DR, Niemczura WP. (1989) Suicide inactivation of lactoperoxidase by 3-amino-1,2,4-triazole. *Chem. Res. Toxicol.* 2;100-103.

Doerge DR, Takazawa RS. (1990) Mechanism of thyroid peroxidase inhibition by ethylenethiourea. *Chem. Res. Toxicol.* 3;98-101.

Domann FE, Mitchen JM, Clifton KH. (1990) Restoration of thyroid function after total thyroidectomy and quantitative thyroid cell transplantation. *Endocrinology* 127;2673-2678.

Donghi R, Sozzi G, Pierotti MA, Biunno I, Miozzo M, Fusco A, Grieco M, Santoro M, Vecchio G, Spurr NK, Della Porta G. (1989) The oncogene associated with human papillary thyroid carcinoma (PTC) is assigned to chromosome 10 q11-q12 in the same region as multiple endocrine neoplasia type 2A (MEN2A). *Oncogene* 4;521-523.

Dugrillon A, Gärtner R. (1995) δ -Iodolactones decrease epidermal growth factor-induced proliferation and inositol-1,4,5-triphosphate generation in porcine thyroid follicles – a possible mechanism of growth inhibition by iodide. *Eur. J. Endocrinol.* 132;735-743.

- Dumont JE, Lamy F, Maenhaut C, Roger RP. (1992) Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. *Physiol. Rev.* 72;667-697.
- Eelkman Rooda SJ, Otten MH, van Loon MAC, Kaptein E, Visser TJ. (1989) Metabolism of triiodothyronine in rat hepatocytes. *Endocrinology* 125;2187-2197.
- Eggo MC, Bachrach LK, Burrow GN. (1990) Interaction of TSH-insulin, and insulin-like growth factors in regulating thyroid growth and function. *Growth Factors* 2;99-109.
- Emerson CH, Lew R, Braverman LE, De Vito WJ. (1989) Serum thyrotropin concentrations are more highly correlated with serum triiodothyronine concentrations than with serum thyroxine concentrations in thyroid hormone-infused thyroidectomized rats. *Endocrinology* 124;2415-2418.
- Esapa C, Foster S, Johnson S, Jameson JL, Kendall-Taylor P, Harris PE. (1997) G protein and thyrotropin receptor mutations in thyroid neoplasia. *J. Clin. Endocrinol. Metab.* 82;493-496.
- Fagin JA. (1994) Molecular genetics of human thyroid neoplasms. *Ann. Rev. Med.* 45;45-52.
- Farid NR, Shi Y, Zou M. (1994) Molecular basis of thyroid cancer. *Endocrine Rev.* 15;202-232.
- Filetti S, Rapoport B. (1983) Evidence that organic iodine attenuates the adenosine 3',5'-monophosphate response to thyrotropin stimulation in thyroid tissue by an action at or near the adenylate cyclase catalytic unit. *Endocrinology* 113;1608-1615.
- Filetti S, Rapoport B. (1984) Autoregulation by iodine of thyroid protein synthesis: influence of iodine on amino-acid uptake in cultured thyroid cells. *Endocrinology* 114;1379-1384.
- Filetti S, Belfiore A, Amir SM, Daniels GH, Ippolito O, Vigneri R, Ingbar SH. (1988) The role of thyroid-stimulating antibodies of Graves' disease in differentiated thyroid cancer. *N. Engl. J. Med.* 318;753-759.
- Franceschi S, Levi F, Negri E, Fassina A, La Vecchia C. (1991) Diet and thyroid cancer: a pooled analysis of four European case-control studies. *Intl. J. Cancer.* 48;395-398.
- Franceschi S, Boyle P, Maisonneuve P, La Vecchia C, Burt AD, Kerr DJ, MacFarlane GJ. (1993) The epidemiology of thyroid carcinoma. *Crit. Rev. Oncogen.* 4;25-52.
- Franklyn JA, Lynam T, Docherty K, Ramsden DB, Sheppard MC. (1986) Effect of hypothyroidism on pituitary cytoplasmic concentrations of messenger RNA encoding thyrotrophin β and α subunits, prolactin and growth hormone. *J. Endocrinol.* 108;43-47.
- Fugazzola L, Pilotti S, Pinchera A, Vorontsova TV, Mondellini P, Bongarzone I, Greco A, Astakhova L, Butti MG, Demidchik EP, Pacini F, Pierotti MA. (1995) Oncogenic

rearrangements of the *RET* proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. *Cancer Res.* 55;5617-5620.

Fujimoto J, Brenner-Gati L. (1992) Protein kinase-C activation during thyrotropin-stimulated proliferation of rat FRTL-5 thyroid cells. *Endocrinology* 130;1587-1592.

Gärtner R, Dugrillon A, Bechtner G. (1990) Evidence that thyroid growth autoregulation is mediated by an iodolactone. *Acta Med. Austriaca* 17;Suppl. 1:124-126.

Gerard CM, Lefort A, Christophe D, Libert F, Van Sande J, Dumont JE, Vassart G. (1989) Control of thyroperoxidase and thyroglobulin transcription by cAMP: evidence for distinct regulatory mechanisms. *Molec. Endocrinol.* 3;2110-2118.

Goepfert H, Callender DL. (1994) Differentiated thyroid cancer - papillary and follicular carcinomas. *Am. J. Otolaryngol.* 15;167-179.

Groch KM, Clifton KH. (1992) The plateau phase rat goiter contains a sub-population of TSH-responsive follicular cells capable of proliferation following transplantation. *Acta Endocrinol.* 126;85-96.

Hawkins MM, Kingston JE. (1988) Malignant thyroid tumours following childhood cancer. *Lancet* 2;804.

Herrmann MA, Hay ID, Bartlett DH, Ritland SR, Dahl RJ, Grant CS, Jenkins RB. (1991) Cytogenetic and molecular genetic studies of follicular and papillary thyroid cancers. *J. Clin. Invest.* 88;1596-1604.

Hiasa Y, Kitahori Y, Kato Y, Ohshima M, Konishi N, Shimoyama T, Sakaguchi Y, Hashimoto H, Minami S, Murata Y. (1987) Potassium perchlorate, potassium iodide, and propylthiouracil: promoting effect on the development of thyroid tumors in rats treated with N-bis(2-hydroxypropyl)-nitrosamine. *Jpn. J. Cancer Res.* 78;1335-1340.

Hiasa Y, Kitahori Y, Kitamura M, Nishioka H, Yane K, Fukumoto M, Ohshima M, Nakaoka S, Nishii S. (1991) Relationships between serum thyroid stimulating hormone levels and development of thyroid tumors in rats treated with N-bis-(2-hydroxypropyl)-nitrosamine. *Carcinogenesis* 12;873-877.

Hiasa Y, Kitahori Y, Konishi N, Ohshima M. (1992) Chemical carcinogenesis in the thyroid gland. *Toxicol. Lett.* 64/65;389-395.

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF. (1989) Thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.* 12;629-697.

Hoelting T, Siperstein AE, Clark OH, Duh Q-Y. (1994) Epidermal growth factor enhances proliferation, migration, and invasion of follicular and papillary thyroid cancer *in vitro* and *in vivo*. *J. Clin. Endocrinol. Metab.* 79;401-408.

Holm L-E, Wiklund KE, Lundell GE, Bergman NA, Bjelkengren G, Cederquist ES, Ericsson U-B, Larsson L-G, Lidberg ME, Lindberg RS, Wicklund HV, Boice JD. (1988) Thyroid cancer after diagnostic doses of iodine-131: a retrospective cohort study. *J. Natl. Cancer Inst.* 80;1132-1138.

Holm L-E, Hall P, Wiklund K, Lundell G, Berg G, Bjelkengren G, Cederquist E, Ericsson U-B, Hallquist A, Larsson L-G, Lidberg M, Lindberg S, Tennvall J, Wicklund H, Boice JD. (1991) Cancer risk after iodine-131 for hyperthyroidism. *J. Natl. Cancer Inst.* 83;1072-1077.

Hölting T, Zielke A, Siperstein AE, Clark OH, Duh Q-Y. (1994) Transforming growth factor- β 1 is a negative regulator for differentiated thyroid cancer: studies of growth migration, invasion, and adhesion of cultured follicular and papillary thyroid cancer cell lines. *J. Clin. Endocrinol. Metab.* 79;806-813.

Horvath E, Lloyd RV, Kovacs K. (1990) Propylthiouracyl-induced hypothyroidism results in reversible transdifferentiation of somatotrophs into thyroidectomy cells. A morphologic study of the rat pituitary including immunoelectron microscopy. *Lab. Invest.* 63;511-520.

Ito T, Seyama T, Mizuno T, Tsuyama N, Hayashi Y, Dohi K, Nakamura N, Akiyama M. (1993) Genetic alterations in thyroid tumor progression: association with p53 gene mutations. *Jpn. J. Cancer Res.* 84;526-531.

Jasani B, Wyllie FS, Wright PA, Lemoine NR, Williams ED, Wynford-Thomas D. (1990) Immunocytochemically detectable TGF- β associated with malignancy in thyroid epithelial neoplasia. *Growth Factors* 2;149-155.

Jenkins RB, Hay ID, Herath JF, Schultz CG, Spurbeck JL, Grant CS, Goellner JR, Dewald GW. (1990) Frequent occurrence of cytogenetic abnormalities in sporadic nonmedullary thyroid carcinoma. *Cancer* 66;1213-1220.

Jhiang SM, Mazzaferri EL. (1994) The ret/PTC oncogene in papillary thyroid carcinoma. *J. Lab. Clin. Med.* 123;331-337.

Kakucska I, Rand W, Lechan RM. (1992) Thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is dependent upon feedback regulation by both triiodothyronine and thyroxine. *Endocrinology* 130;2845-2850.

Kanno J, Matsuoka C, Furuta K, Onodera H, Miyajima H, Maekawa A, Hayashi Y. (1990) Tumor promoting effect of goitrogens on rat thyroid. *Toxicologic Pathol.* 18;239-246.

Kawaoi A, Matsumoto H, Suzuki K, Moriyama S. (1991) Histogenesis of diisopropanolnitrosamine (DIPN)-induced tumors of the rat thyroid gland. *Virchows Archiv. B. Cell Pathol.* 61;49-56.

Kerber RA, Till JE, Simon SL, Lyon JL, Thomas DC, Preston-Martin S, Rallison ML, Lloyd RD, Stevens W. (1993) A cohort study of thyroid disease in relation to fallout from nuclear weapons testing. *J. Am. Med. Assoc.* 270;2076-2082.

Kitahori Y, Hiasa Y, Katoh Y, Konishi N, Ohshima M, Hashimoto H, Minami S, Sakaguchi Y. (1988) Promotive effect of 4,4'-methylenebis(N,N-dimethyl)benzenamine on N-bis(2-hydroxypropyl)nitrosamine-induced thyroid tumors in Wistar rats. *Cancer Lett.* 40;275-281.

Kitahori Y, Ohshima M, Matsuki H, Konishi N, Hashimoto H, Minami S, Thamavit W, Hiasa Y. (1989) Promoting effect of 2,4-diaminoanisole sulfate on rat thyroid carcinogenesis. *Cancer Lett.* 45;115-121.

Kitahori Y, Naito H, Konishi N, Ohnishi T, Shirai T, Hiasa Y. (1995) Frequent mutations of *Ki-ras* codon 12 in N-bis(2-hydroxypropyl)-nitrosamine-initiated thyroid, kidney and lung tumors in Wistar rats. *Cancer Lett.* 96;155-161.

Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM. (1995) High prevalence of *RET* rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. *Oncogene* 11;2459-2467.

Koller KJ, Wolff RS, Warden MK, Zoeller RT. (1987) Thyroid hormones regulate levels of thyrotropin-releasing-hormone mRNA in the paraventricular nucleus. *Proc. Natl. Acad. Sci. USA* 84;7329-7333.

Laugwitz K-L, Allgeier A, Offermanns S, Spicher K, Van Sande J, Dumont JE, Schultz G. (1996) The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. *Proc. Natl. Acad. Sci. USA* 93;116-120.

Laurent E, Mockel J, van Sande J, Graff I, Dumont JE. (1987) Dual activation by thyrotropin of the phospholipase C and cyclic AMP cascades in human thyroid. *Molec. Cell. Endocrinol.* 52;273-278.

Leer LM, Cammenga M, van der Vorm ER, Vijlder JJM. (1991) Methimazole increases thyroid-specific mRNA concentration in human thyroid cells and FRTL-5 cells. *Molec. Cell. Endocrinol.* 78;221-228.

Lemoine NR, Mayall ES, Williams ED, Thurston V, Wynford-Thomas D. (1988) Agent-specific *ras* oncogene activation in rat thyroid tumours. *Oncogene* 3;541-544.

Lemoine NR, Mayall ES, Wyllie FS, Williams ED, Goyns M, Stringer B, Wynford-Thomas D. (1989) High frequency of *ras* oncogene activation in all stages of human thyroid tumorigenesis. *Oncogene* 4;159-164.

Leonard JL, Visser TJ. (1986) Biochemistry of deiodination. In: *Thyroid Hormone Metabolism*. Hennemann G (ed). Marcel Dekker, New York, pp 189-222.

Levi F, Franceschi S, La Vecchia C, Negri E, Gulie C, Duruz G, Scazziga B. (1991) Previous thyroid disease and risk of thyroid cancer in Switzerland. *Eur. J. Cancer* 27;85-88.

Libert F, Lefort A, Gerard C, Parmentier M, Perret J, Ludgate M, Dumont JE, Vassart G. (1989) Cloning, sequencing and expression of the human (TSH) receptor: evidence for binding of autoantibodies. *Biochem. Biophys. Res. Commun.* 165;1250-1255.

Littlefield NA, Gaylor DW, Blackwell BN, Allen RR. (1989) Chronic toxicity/carcinogenicity studies of sulphamethazine in B6C3F₁ mice. *Food Chem. Toxicol.* 27;455-463.

Littlefield NA, Sheldon WG, Allen R, Gaylor DW. (1990) Chronic toxicity/carcinogenicity studies of sulphamethazine in Fischer 344/N rats: two-generation exposure. *Food Chem. Toxicol.* 28;157-167.

Logan A, Black AG, Gonzalez A-M, Buscaglia M, Sheppard MC. (1992) Basic fibroblast growth factor: an autocrine mitogen of rat thyroid follicular cells. *Endocrinology* 130;2363-2372.

Logan A, Smith C, Becks GP, Gonzalez AM, Phillips ID, Hill DJ. (1994) Enhanced expression of transforming growth factor- β 1 during thyroid hyperplasia in rats. *J. Endocrinol.* 141;45-57.

LoPresti JS, Nicoloff JT. (1994) 3,5,3'-triiodothyronine (T₃) sulfate: a major metabolite in T₃ metabolism in man. *J. Clin. Endocrinol. Metab.* 78;688-692.

Maenhaut C, Lefort A, Libert F, Parmentier M, Raspé E, Roger P, Corvilain B, Laurent E, Reuse S, Mockel J, Lamy F, Van Sande J, Dumont JE. (1990) Function, proliferation and differentiation of the dog and human thyrocyte. *Hormone Metab. Res. Suppl.* 23;51-61.

Marion S, Ropars A, Ludgate M, Braun JM, Charreire J. (1992) Characterization of monoclonal antibodies to the human thyrotropin receptor. *Endocrinology* 130;967-975.

Mazzaferrri EL. (1990) Thyroid cancer and Graves' disease. *J. Clin. Endocrinol. Metab.* 70;826-828.

McClain RM. (1989) The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: implications for thyroid gland neoplasia. *Toxicologic Pathol.* 17;294-306.

McClain RM. (1995) The use of mechanistic data in cancer risk assessment: case example – sulfonamides. In: *Low Dose Extrapolation of Cancer Risks: Issues and Perspectives*. Olin S, Farland W, Park C, Rhomberg L, Scheuplein R, Starr T, Wilson J, (eds). ILSI Press, Washington, DC, pp 163-173.

McClain RM, Posch RC, Bosakowski T, Armstrong JM. (1988) Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. *Toxicol. Appl. Pharmacol.* 94;254-265.

McClain RM, Levin AA, Posch R, Downing JC. (1989) The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicol. Appl. Pharmacol.* 99;216-228.

Minuto F, Barreca A, del Monte P, Cariola G, Torre GC, Giordano G. (1989) Immunoreactive insulin-like growth factor I (IGF-I) and IGF-I-binding protein content in human thyroid tissue. *J. Clin. Endocrinol. Metab.* 68;621-626.

Mirell CJ, Yanagisawa M, Lau R, Pekary AE, Chin WW, Hershman JM. (1987) Influence of thyroidal status on pituitary content of thyrotropin β and α -subunit, growth hormone, and prolactin messenger ribonucleic acids. *Molec. Endocrinol.* 1;408-412.

Misrahi M, Loosfelt H, Atger M, Sar S, Guiochon-Mantel A, Milgrom E. (1990) Cloning, sequencing and expression of human TSH receptor. *Biochem. Biophys. Res. Commun.* 166;394-403.

Mitsumori K, Onodera H, Takahashi M, Shimo T, Yasuhara K, Kitaura K, Takahashi M, Hayashi Y. (1995) Effect of thyroid stimulating hormone on the development and progression of rat thyroid follicular cell tumors. *Cancer Lett.* 92;193-202.

Mizukami Y, Hashimoto T, Nonomura A, Michigishi T, Nakamura S, Noguchi M, Matsukawa S. (1994) Immunohistochemical demonstration of thyrotropin (TSH)-receptor in normal and diseased human thyroid tissues using monoclonal antibody against recombinant human TSH-receptor protein. *J. Clin. Endocrinol. Metab.* 79;616-619.

Mockel J, Laurent E, Lejeune C, Dumont JE. (1991) Thyrotropin does not activate the phosphatidylinositol bisphosphate hydrolyzing phospholipase C in the dog thyroid. *Molec. Cell. Endocrinol.* 82;221-227.

Mori M, Naito M, Watanabe H, Takeichi N, Dohi K, Ito A. (1990) Effects of sex difference, gonadectomy, and estrogen on N-methyl-N-nitrosourea induced rat thyroid tumors. *Cancer Res.* 50;7662-7667.

Morris JC, Ranganathan G, Hay ID, Nelson RE, Jiang N-S. (1988) The effects of transforming growth factor- β on growth and differentiation of the continuous rat thyroid follicular cell line, FRTL-5. *Endocrinology* 123;1385-1394.

Murthy ASK, Russfield AB, Snow GJ. (1985) Effect of 4,4'-oxydianiline on the thyroid and pituitary glands of F344 rats: a morphologic study with the use of the immunoperoxidase method. *J. Natl. Cancer Inst.* 74;203-208.

Nagayama Y, Rapoport B. (1992) The thyrotropin receptor 25 years after its discovery: new insight after its molecular cloning. *Molec. Endocrinol.* 6;145-156.

Nagayama Y, Kaufman KD, Seto P, Rapoport B. (1989) Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. *Bochem. Biophys. Res. Commun.* 165;1184-1190.

Nakamura T, Yana I, Kobayashi T, Shin E, Karakawa K, Fujita S, Miya A, Mori T, Nishisho I, Takai S. (1992) p53 Gene mutations associated with anaplastic transformation of human thyroid carcinomas. *Jpn. J. Cancer Res.* 83;1293-1298.

Namba H, Gutman RA, Matsuo K, Alvarez A, Fagin JA. (1990) H-ras protooncogene mutations in human thyroid neoplasms. *J. Clin. Endocrinol. Metab.* 71;223-229.

National Toxicology Program (NTP). (1988) Toxicology and carcinogenesis studies of malonaldehyde, sodium salt (3-hydroxy-2-propenal, sodium salt) (CAS No. 24382-04-5) in F344/N and B6C3F₁ mice (gavage studies). National Toxicology Program. NTP TR 331, NIH Publication. No. 89-2587.

Nikiforov Y, Gnepp DR. (1994) Pediatric cancer after the Chernobyl disaster. Pathomorphologic study of 84 cases (1991-1992) from the republic of Belarus. *Cancer* 74;748-766.

Nikiforov Y, Gnepp DR, Fagin JA. (1996) Thyroid lesions in children and adolescents after the Chernobyl disaster: implications for the study of radiation tumorigenesis. *J. Clin. Endocrinol. Metab.* 81;9-14.

Nilsson M. (1995) Actions of epidermal growth factor and its receptor in the thyroid. *Trends Endocrinol. Metab.* 6;175-182.

Ossendorp FA, Bruning PF, Schuurung EMD, Van Den Brink JAM, Van der Heide D, De Vijlder JJM, de Bruin TWA. (1990) Thyrotropin dependent and independent thyroid cell lines selected from FRTL-5 derived tumors grown in nude mice. *Endocrinology* 127;419-430.

O'Sullivan C, Barton CM, Staddon SL, Brown CL, Lemoine NR. (1991) Activating point mutations of the *gsp* oncogene in human thyroid adenomas. *Molec. Carcinogen.* 4;345-349.

Parmentier M, Libert F, Maenhaut C, Lefort A, Gérard C, Perret J, Van Sande J, Dumont JE, Vassart G. (1989) Molecular cloning of the thyrotropin receptor. *Science* 246;1620-1622.

Paschke R, Vassart G, Ludgate M. (1995) Current evidence for and against the TSH receptor being the common antigen in Graves' disease and thyroid associated ophthalmopathy. *Clin. Endocrinol.* 42;565-569.

Pekary AE, Berg L, Santini F, Chopra I, Hershman JM. (1994) Cytokines modulate type I iodothyronine deiodinase mRNA levels and enzyme activity in FRTL-5 rat thyroid cells. *Molec. Cell. Endocrinol.* 101;R31-R35.

Pereira A, Braekman J-C, Dumont JE, Boeynaems J-M. (1990) Identification of a major iodolipid from the horse thyroid gland as 2-iodohexadecanal. *J. Biol. Chem.* 265;17018-17025.

Peter HJ, Gerber H, Studer H, Smeds S. (1985) Pathogenesis of heterogeneity in human multinodular goiter. *J. Clin. Invest.* 76;1990-2002.

Pettersson B, Adami H-O, Wilander E, Coleman MP. (1991) Trends in thyroid cancer incidence in Sweden, 1958-1981, by histopathologic type. *Intl. J. Cancer* 48;28-33.

Pettersson B, Coleman MP, Ron E, Adami H-O. (1996) Iodine supplementation in Sweden and regional trends in thyroid cancer incidence by histopathologic type. *Intl. J. Cancer* 65;13-19.

Pisarev MA, Chazenbalk GD, Valsecchi RM, Burton G, Krawiec L, Monteagudo E, Juvenal GJ, Boado RJ, Chester HA. (1988) Thyroid autoregulation. Inhibition of goiter growth and of cyclic AMP formation in rat thyroid by iodinated derivatives of arachidonic acid. *J. Endocrinol. Invest.* 11;669-674.

Rees Smith B, McLachlan SM, Furmaniak J. (1988) Autoantibodies to the thyrotropin receptor. *Endocrine Rev.* 9;106-121.

Ridgway EC. (1992) Clinician's evaluation of a solitary thyroid nodule. *J. Clin. Endocrinol. Metab.* 74;231-235.

Roger PP. (1996) Thyrotropin-dependent transforming growth factor beta expression in thyroid gland. *Eur. J. Endocrinol.* 134;269-271.

Roger P, Taton M, Van Sande J, Dumont JE. (1988) Mitogenic effects of thyrotropin and adenosine 3',5'-monophosphate in differentiated normal human thyroid cells in vitro. *J. Clin. Endocrinol. Metab.* 66;1158-1165.

Ron E, Modan B, Preston D, Alfandary E, Stovall MS, Boice JD. (1989) Thyroid neoplasia following low-dose radiation in childhood. *Radiat. Res.* 120;516-531.

Rondeel JMM, de Greef WJ, Klootwijk W, Visser TJ. (1992) Effects of hypothyroidism on hypothalamic release of thyrotropin-releasing hormone in rats. *Endocrinology* 130;651-656.

Roque L, Castedo S, Gomes P, Soares P, Clode A, Soares J. (1993a) Cytogenetic findings in 18 follicular thyroid adenomas. *Cancer Genet. Cytogenet.* 67;1-6.

Roque L, Gomes P, Correia C, Soares P, Soares J, Castedo S. (1993b) Thyroid nodular hyperplasia: chromosomal studies in 14 cases. *Cancer Genet. Cytogenet.* 69;31-34.

Rutgers M, Heusdens FA, Bonthuis F, de Herder WW, Hazenberg MP, Visser TJ. (1989a) Enterohepatic circulation of triiodothyronine (T_3) in rats: importance of the microflora for the liberation and reabsorption of T_3 from biliary T_3 conjugates. *Endocrinology* 125;2822-2830.

Rutgers M, Pigman IGJ, Bonthuis F, Doctor R, Visser TH. (1989b) Effects of propylthiouracil on the biliary clearance of thyroxine (T_4) in rats; decreased excretion of 3,5,3'-triiodothyronine glucuronide and increased excretion of 3,3,5'-triiodothyronine glucuronide and T_4 sulfate. *Endocrinology* 125;2175-2186.

Said S, Schlumberger M, Suarez HG. (1994) Oncogenes and anti-oncogenes in human epithelial thyroid tumours. *J. Endocrinol. Invest.* 17;371-379.

Saji M, Akamizu T, Sanchez M, Obici S, Avvedimento E, Gottesman ME, Kohn LD. (1992) Regulation of thyrotropin receptor gene expression in rat FRTL-5 thyroid cells. *Endocrinology* 130;520-533.

Samuels MH, Wiermann ME, Wang C, Ridgway EC. (1989) The effect of altered thyroid status on pituitary hormone messenger ribonucleic acid concentrations in the rat. *Endocrinology* 124;2277-2282.

Santisteban P, Kohn LD, Di Lauro R. (1987) Thyroglobulin gene expression is regulated by insulin and IGF-I as well as thyrotropin in FRTL-5 thyroid cells. *J. Biol. Chem.* 262;4048-4052.

Segerson TP, Kauer J, Wolfe HC, Mobtaker H, Wu P, Jackson IMD, Lechan RM. (1987) Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* 238;78-80.

Shi Y, Zou M, Schmidt H, Juhasz F, Stensky V, Robb D, Farid NR. (1991) High rates of *ras* codon 61 mutation in thyroid tumors in an iodide-deficient area. *Cancer Res.* 51;2690-2693.

Shimo T, Mitsumori K, Onodera H, Yasuhara K, Takahashi M, Takahashi M, Ueno Y, Hayashi Y. (1994) Time course observation of thyroid proliferative lesions and serum TSH levels in rats treated with thiourea after DHPN initiation. *Cancer Lett.* 85;141-149.

Smeds S, Peter HJ, Jörtso E, Gerber H, Studer H. (1987) Naturally occurring clones of cells with high intrinsic proliferation potential within the follicular epithelium of mouse thyroids. *Cancer Res.* 47;1646-1651.

Spencer CA. (1996) Dynamics of thyroid hormone suppression of serum thyrotropin: an invited commentary. *Eur. J. Endocrinol.* 135;285-286.

Studer H, Derwahl M. (1995) Mechanisms of nonneoplastic endocrine hyperplasia – a changing concept: a review focused on the thyroid gland. *Endocrine Rev.* 16;411-426.

Studer H, Peter HJ, Gerber H. (1989) Natural heterogeneity of thyroid cells: the basis for understanding thyroid function and nodular goiter growth. *Endocrinol. Rev.* 10;125-135.

Suarez HG, du Villard JA, Caillou B, Schlumberger M, Parmentier C, Monier R. (1991) *gsp* Mutations in human thyroid tumours. *Oncogene* 6;677-679.

Takahashi S-I, Conti M, Van Wyk JJ. (1990) Thyrotropin potentiation of insulin like growth factor-I dependent deoxyribonucleic acid synthesis in FRTL-5 cells: mediation by autocrine amplification factors. *Endocrinology* 126;736-745.

Takayama S, Aihara K, Onodera T, Akimoto T. (1986) Antithyroid effects of propylthiouracil and sulfamonomethoxine in rats and monkeys. *Toxicol. Appl. Pharmacol.* 82;191-199.

Taurog A, Dorris ML. (1988) Propylthiouracil and methimazole display contrasting pathways of peripheral metabolism in both rat and human. *Endocrinology* 122;592-601.

Taurog A, Dorris ML. (1989) A reexamination of the proposed inactivation of thyroid peroxidase in the rat thyroid by propylthiouracil. *Endocrinology* 124;3038-3042.

Taurog A, Dorris ML, Guziec FS, Uetrecht JP. (1989) Metabolism of ³⁵S- and ¹⁴C-labeled propylthiouracil in a model *in vitro* system containing thyroid peroxidase. *Endocrinology* 124;3030-3037.

Taylor AH, Millatt LJ, Whitley GS, Johnstone AP, Nussey SS. (1993) The effect of basic fibroblast growth factor on the growth and function of human thyrocytes. *J. Endocrinol.* 136;339-344.

Thomas GA, Williams ED. (1991) Evidence for and possible mechanisms of non-genotoxic carcinogenesis in the rodent thyroid. *Mutation Res.* 248;357-370.

Tucker MA, Coleman CN, Cox RS, Vargese A, Rosenberg SA. (1988) Risk of second malignancies after treatment for Hodgkin's disease. *N. Engl. J. Med.* 318;76-81.

Tucker MA, Morris Jones PH, Boice JD, Robison LL, Stone BJ, Stovall M, Jenkin RDT, Lubin JH, Baum ES, Siegel SE, Meadows AT, Hoover RN, Fraumeni JF. (1991) Therapeutic radiation at a young age is linked to secondary thyroid cancer. *Cancer Res.* 51;2885-2888.

Uyttersprot N, Van Sande J, Dumont JE. (1995) Thyroid adenoma, Gs α expression and the cyclic adenosine monophosphate mitogenic cascade: a complex relationship. *J. Clin. Endocrinol. Metab.* 80;1518-1520.

Van Heuverswyn B, Leriche A, Van Sande J, Dumont JE, Vassart G. (1985) Transcriptional control of thyroglobulin gene expression by cyclic AMP. *FEBS Lett.* 188;192-196.

Vassart G, Dumont JE. (1992) The thyrotropin receptor and the regulation of thyrocyte function and growth. *Endocrine Rev.* 13;596-611.

Viglietto G, Chiapetta G, Martinez-Tello FJ, Fukunaga FH, Tallini G, Rigopoulou D, Visconti R, Mastro A, Santoro M, Fusco A. (1995) RET/PTC oncogene activation is an early event in thyroid carcinogenesis. *Oncogene* 11;1207-1210.

Visser TJ, Kaptein E, Terpstra OT, Krenning EP. (1988) Deiodination of thyroid hormone by human liver. *J. Clin. Endocrinol. Metab.* 67;17-24.

Visser TJ, van Buuren JCJ, Rutgers M, Eelkman Rooda SJ, de Herder WW. (1990) The role of sulfation in thyroid hormone metabolism. *Trends Endocrine Metab.* 1;211-218.

Visser TJ, Kaptein E, van Toor HR, van Raaij JAGM, van den Berg K, Joe CTT, van Engelen JGM, Brouwer A. (1993) Glucuronidation of thyroid hormone in rat liver: effects of *in vivo* treatment with microsomal enzyme inducers and *in vitro* assay conditions. *Endocrinology* 133;2177-2186.

Wang Z, Boice JD, Wei L, Beebe GW, Zha Y, Kaplan MM, Tao Z, Maxon HR, Zhang S, Schneider AB, Tan B, Wesseler TA, Chen D, Ershow AG, Kleinerman RA, Littlefield LG, Preston D. (1990) Thyroid nodularity and chromosome aberrations among women in areas of high background radiation in China. *J. Natl. Cancer Inst.* 82;478-485.

Watanabe H, Tanner MA, Domann FE, Gould MN, Clifton KH. (1988) Inhibition of carcinoma formation and of vascular invasion in grafts of radiation-initiated thyroid clonogens by unirradiated thyroid cells. *Carcinogenesis* 9;1329-1335.

Williams D. (1996) Thyroid cancer and the Chernobyl accident. *J. Clin. Endocrinol. Metab.* 81;6-7.

Williams DW, Williams ED, Wynford-Thomas D. (1988) Loss of dependence on IGF-1 for proliferation of human thyroid adenoma cells. *Br. J. Cancer* 57;535-539.

Williams DW, Williams ED, Wynford-Thomas D. (1989) Evidence for autocrine production of IGF-1 in human thyroid adenomas. *Molec. Cell. Endocrinol.* 61;139-143.

Wolff J. (1989) Excess iodide inhibits the thyroid by multiple mechanisms. *Adv. Exp. Med. Biol.* 261;211-244.

Wood DF, Franklyn JA, Docherty K, Ramsden DB, Sheppard MC. (1987) The effect of thyroid hormones on growth hormone gene expression *in vivo* in rats. *J. Endocrinol.* 112;459-463.

Wright PA, Lemoine NR, Mayall ES, Wyllie FS, Hughes D, Williams ED, Wynford-Thomas D. (1989) Papillary and follicular thyroid carcinomas show a different pattern of ras oncogene mutation. *Cancer.* 60;576-577.

Wright PA, Williams ED, Lemoine NR, Wynford-Thomas D. (1991) Radiation-associated and "spontaneous" human thyroid carcinomas show a different pattern of ras oncogene mutation. *Oncogene.* 6;471-473.

Wynford-Thomas D. (1993) Molecular basis of epithelial tumorigenesis: the thyroid model. *Crit. Rev. Oncogen.* 4;1-23.

Wynford-Thomas D, Stringer BMJ, Williams ED. (1982) Desensitisation of rat thyroid to the growth-stimulating action of TSH during prolonged goitrogen administration. Persistence of refractoriness following withdrawal of stimulation. *Acta Endocrinol.* 101;562-569.