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Effects of 3, 5, 6-Trichloro-2-Pyridinol on Thyroid Function

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EFFECTS OF 3,5,6-TRICHLORO-2-PYRIDINOL ON THYROID FUNCTION

by

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Charles Franklin Luke

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Effects of 3,5,6-trichloro-2-pyridinol

On Thyroid Function

by

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3,5,6-trichloro-2-pyridinol, when administered in the diet, increases the feed efficiency (the ratio of weight gain to feed consumed) in various species of domestic animals and also appears to be retained in the liver at low concentrations, possibly by binding to a specific macromolecule. Because of its structural similarities to the outer ring of the thyroid hormones, trichloropyridinol and three structural analogs (2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, or 4-iodo-2,5-dichlorophenol) were tested for ability to compete *in vitro* with 3,5,3`-triiodothyronine (T_3) for nuclear receptors specific for the thyroid hormones. All four of the halogenated compounds were found to be weakly competitive for the receptor, indicating a possible anti-thyroid effect.

Weanling male rats were fed diets containing 5, 50, and 500 ppm trichloropyridinol for 30, 60, and 90 days. Other groups received a diet containing 200 and 2000 ppm 2-thiouracil, a known thyroid toxicant. Both chemicals significantly reduced serum thyroxine (T_4) levels in a dose-related manner. It was of interest that trichloropyridinol was about as potent as thiouracil in suppressing serum T_4 levels. Serum T_3 and 3,3`5`-triiodothyronine (rT_3) levels were depressed by thiouracil, but generally not with trichloropyridinol. Nuclear T_3 binding capacity was not changed in the liver of rats ingesting trichloropyridinol. Body weight, feed efficiency, and organ weights and histology were not significantly altered by the chronic ingestion of trichloropyridinol.

In conclusion, the effects of trichloropyridinol upon animal growth and feed efficiency may involve various mechanisms surrounding thyroid hormone expression including reduction of serum T_4 levels and competition with T_3 for the nuclear receptor.

(72 pages)

INTRODUCTION

3,5,6-trichloro-2-pyridinol is a mammalian metabolite of two widely used organophosphate insecticides: chlorpyrifos (Dursban) and chlorpyrifos-methyl (Dowco 214). Various insecticides which are capable of being metabolized to halogenated phenols, such as leptophos (O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate) and ronnel (O,O-dimethyl O-(2,4,5-trichlorophenyl)phosphorothioate), increase feed efficiency (gram of weight gain per gram of feed consumed). Leptophos, fed to dairy cattle as residue on corn silage, increased body weight gains and decreased milk production. (Johnson et al., 1971). Ronnel increased the feed efficiency in cattle (Rumsey et al., 1975; Riley and Ware, 1977; Wooden and Algeo, 1977; Thomas and Ware, 1978; Rumsey, 1979; Rumsey et al., 1981) and rats (Trankina et al., 1979; 1981; 1982). In addition, ronnel increased plasma T_4 (thyroxine) concentration but not T_3 (3,3`,5-triiodothyronine) in steers, indicating an alteration of thyroid function (Bitman and Rumsey, 1980).

Chronic ingestion of trichloropyridinol increased the feed efficiency in several species of animals (Hymas, T., personal communication) and altered the histological structure but not the weight of the thyroid gland in rats (Beck, 1976). The thyroid gland secretes hormones which produce a

calorigenic effect by accelerating catabolism (Sestoft, 1979). Lower serum levels of thyroid hormones reduce catabolism and increase feed efficiency. For example, partially thyroidectomized cattle had better weight gains than normal cattle (Bullard and Andrews, 1943).

Because of these findings and because of the structural similarity of trichloropyridinol to the outer ring of T_4 and T_3, we hypothesized that trichloropyridinol may increase feed efficiency by altering serum thyroid hormone levels or by interacting with the hepatic nuclear T_3 receptor.

In this study, trichloropyridinol and three structural analogs competed with T_3 for specific nuclear receptors in vitro. Because of this finding, the serum T_4, T_3, and rT_3 (3,3',5'-triiodothyronine) levels and the T_3 binding capacity in isolated hepatic nuclei were measured from rats that ingested feed containing trichloropyridinol for 30, 60, and 90 days. Other groups of rats fed thiouracil, a well known inhibitor of normal thyroid hormone synthesis, served as positive controls.

REVIEW OF LITERATURE

The thyroid gland secretes two classes of hormones: (1) calcitonin which regulates calcium metabolism and (2) the "classical" thyroid hormones, T_4 and T_3. In this dissertation, the term thyroid hormones refers strictly to the latter group.

Upon proper stimulus, the thyroid gland synthesizes, stores, and secretes the thyroid hormone. Thyroid hormone synthesis begins with the thyroid cells forming thyroglobulin which is then secreted into the follicular lumen where it is iodinated by thyroid peroxidase resulting in monoiodotyrosine and diiodotyrosine residues. Two of the tyrosine residues are coupled together producing T_4, T_3, diiodothyronine, and monoiodothyronine residues, still part of the thyroglobulin molecule. This protein is taken back into the thyroid cell and hydrolyzed freeing T_4 and T_3 which is secreted into the blood (Haynes and Murad, 1981).

In man, approximately 15% of circulating T_3 comes from direct thyroidal secretion with the remaining from deiodination of T_4 in peripheral tissues (Surks et al., 1973). In the rat, about 50% is from direct secretion with an equal amount coming from peripheral deiodination. T_4 can be deiodinated at the 5` location giving T_3 or at the 5 location giving rT_3 (Figure 1). The formation of T3 is an activation

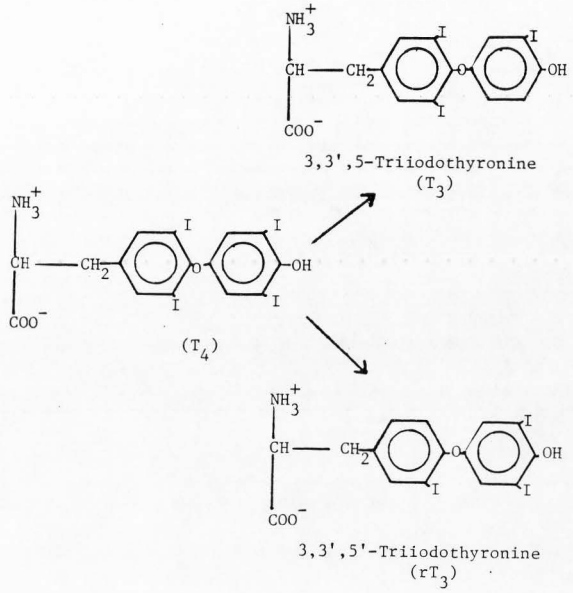

Fig. 1. Schematic diagram of the metabolic conversion of thyroxine (T_4) to 3,3',5-triiodothyronine (T_3) and 3,3',5'-triiodothyronine (rT_3).

reaction because T_3 is a more potent or biologically active thyroid hormone than T_4; however, the formation of rT_3 is a deactivation step because rT_3 is less biologically active than T_4 (Tipperman, 1980).

The majority of the extrathyroidal iodothyronines (~99%) are bound to proteins in the plasma, interstitial fluid, or cellular component (Oppenheimer et al., 1969).

While being transported in the blood, thyroid hormones are bound tightly, but noncovalently, to plasma proteins which protect the hormones from metabolism and excretion. In humans, thyroxine-binding globulin (TBG) is the major carrier of thyroid hormones, followed by thyroxine-binding prealbumin and albumin (Haynes and Murad, 1980). Rats lack TBG; therefore, thyroid hormones are bound mostly to the albumin fraction (Robbins and Rall, 1960).

It is uncertain whether the amount of thyroid hormone available to the cells is only that small fraction that is not bound to plasma proteins, or if the binding is reversible enough that all the serum thyroid hormone in the plasma is readily available. The rate of transcapillary passage of thyroid hormone (Simpson-Morgan and Sutherland, 1976) and the rate of uptake by tissues (Cavalieri and Searle, 1966; Cavalieri et al., 1970) correlates with the unbound rather than with the total or bound fraction of circulating hormone; however, the clearance of various iodothyronines did not correlate with the fraction unbound, indicating that the

binding may be rapidly reversed (Pardridge and Mietus, 1980).

Iodothyronines are transferred into the cell either by facilitated or active transport mechanisms (Cheng et al., 1980; Rao,1981; Maxfield et al., 1981; Halperin and Hinkle, 1982). Once in the cell, they bind to macromolecules found in the various subcellular fractions.

Schadlow et al. (1972) injected rats iv with traceable doses of labelled T_4 and T_3 and various concentrations of unlabelled iodothyronine providing the first evidence that intracellular limited capacity binding sites existed, but were only able to detect these sites in the pituitary. Oppenheimer et al. (1972) found that the nuclear fraction of the liver and kidney also had limited capacity binding sites. Later, limited capacity binding sites were found in the cytosol (Dillmann et al., 1974), plasma membrane (Segel and Ingbar, 1980), and mitochondria (Sterling and Milch, 1975). For more information on the non-nuclear T_3 binding proteins see Sterling (1979) and Barsano and DeGroot (1983).

Nuclear Receptor

The following experimental evidence indicates that the limited capacity binding sites found in the nuclei are true receptors involved in the initiation of thyroid hormone action. Admittedly, none of the evidence in itself is completely conclusive; however, the evidence is very convincing when taken as a whole.

(1) The nuclear binding sites have high affinity and low capacity for thyroid hormones (Oppenheimer et al., 1976; DeGroot and Torresani, 1975; Samuels, 1978; and Baxter et al., 1979). Association constants for T_3 of 5 X $10^{11} M^{-1}$ and binding capacity of 0.5 ng/mg DNA were measured in nuclei from rat liver (Oppenheimer, et al., 1974a). Although high affinity and low capacity are generally associated with hormone receptors, these properties alone are not sufficient nor are they logically necessary characteristics. As proof of the latter point, serum hormone binding proteins have high affinity and low-capacity but are not receptors involved in the initiation of thyroid hormone action (Haynes and Murad, 1980).

(2) The binding sites are non-histone nucleoproteins which indicate they may have a regulatory function. The nuclear involvement in thyroid hormone response was first shown by Tata and Widnell (1966) who found that immediately after a thyroidectomized rat was injected with a single dose of T_3 there was an enhanced incorporation of ^{14}C-orotic acid into nuclear RNA, followed by enhanced RNA polymerase I activity, and then an increased synthesis of protein. Other studies have also indicated the involvement of nuclear function in thyroid hormone response (reviewed in Towle, 1983).

(3) Nuclear binding affinity of various thyroid hormone analogues correlates well with their thyromimetic potency. Injecting animals with trace amounts of radiolabelled T_3

together with graded doses of unlabelled T_3 and various analogues indicated that the ability of these analogues to compete with T_3 for the nuclear binding sites correlate well with their thyromimetic activities (Oppenheimer et al., 1973). This finding was confirmed in in vitro competitive binding studies using isolated nuclei (Koerner et al., 1975; DeGroot and Torresani, 1975) and solubilized nuclear extracts (Latham et al., 1976).

(4) Tissues responsive to thyroid hormones also have a high concentration of the putative nuclear receptor sites. The oxygen consumption of most excised rat tissues varied with the thyroidal status of the animal; however, this was not the case with the brain, spleen, lung, testis, and ovary (Barker and Klitgaard, 1952). The nuclear binding capacities (number of sites per tissue nucleus) were pituitary, 5200; liver, 4000; kidney, 3480; heart, 2600; brain, 1760; spleen, 120; and testis, 16 (Oppenheimer et al., 1974b). Therefore, the tissues in which oxygen consumption was not affected by the concentration of thyroid hormones also had the lowest level of nuclear binding sites.

(5) Patients with apparent clinical resistance to T_3 also exhibited abnormalities in nuclear binding (Bernal et al., 1976).

(6) Nuclear occupancy correlates with cellular response both in tissue culture system and in the intact animal. GH_1 cell cultures, a cell line derived from pituitary tumors of

rats, are capable of producing growth hormone (reviewed in Samuels, 1983). The rate of growth hormone synthesis in this cell line was found to be linearly related to nuclear occupancy (Samuels et al., 1976).

Calorigenic Effect of Thyroid Hormones

Magnus-Levy (1985) found that patients with myxedema had abnormally lower oxygen consumption than to normal individuals. It was later found that tissues from hypothyroid and hyperthyroid patients had lower and higher oxygen consumption, respectively (Barker and Klitgaard, 1952). The calorigenic effect of thyroid hormones is reflected by the rate of oxygen consumption. Gross (1971) found that the rate of oxygen consumption decreased from 3.5×10^{-2} ml O_2/min/g body weight to 2.5×10^{-2} ml O_2/min/g body weight when the thyroid gland was removed from rats. This rate returned to normal with appropriate administration of T_4. The calorigenic effect of thyroid hormones could take place via numerous possible mechanisms (Table 1).

Uncoupling mechanism. The mitochondria from animals treated with large doses of thyroid hormones were found to be uncoupled (Lardy and Feldott, 1951; Maritus and Hess, 1951). During the 1950's, several investigators (eg. Hoch and Lipmann, 1954; Maley and Lardy, 1955) came to the conclusion that the increased rate of uptake was due to the uncoupling of phosphorylation, analogous to the action of

TABLE 1

POSSIBLE MECHANISMS OF CALORIGENESIS
INDUCED BY THYROID HORMONES

1. Uncoupling of Mitochondria
2. Increasing Gluconeogenesis
3. Increasing fat synthesis and degradation at same time (creating a futile fat cycle)
4. Stimulating Na^+/K^+ ATPase activity
5. Increasing the use of α-glycerol phosphate shuttle instead of the malate-aspartate shuttle
6. Producing hyperactivity of the heart

dinitrophenol (DNP). The validity of this theory was challenged in the early 1960's mostly because of the finding of Tata et al.(1963) that the mitochondria from animals treated with a smaller dose of thyroid hormone (approximating normal physiological concentrations) had normal P:O ratios, maintaining normal energy conservation. Other findings according to Gurnsey and Edelman (1983) which challenged the uncoupling mechanism hypothesis included: (1) in vivo biological actions of thyroid hormones did not mimic those of DNP, (2) iodinated compounds that are non-calorigenic in vivo were as active as T_4 in uncoupling oxidative phosphorylation (Tapley and Cooper, 1956), (3) the latent period between the injection of T_4 and the calorigenic action was too long (Briggs et al., 1953; Gross and Leblond, 1947), and (4) instead of having a decreased adenosine triphosphate (ATP) formation and concentration, which would happen if the mitochondria were uncoupled, the ATP concentration was normal or slightly elevated in hyperthyroid tissue (Olsson, 1964; Buccino et al., 1967; Burns and Reddy, 1975; Nishiki et al., 1978).

Energy liberated from the oxidation of nutrients is stored in the form of ATP which is then used to do cellular work. ATP is hydrolyzed to perform various cellular functions such as (1) ion transport; (2) synthesis of proteins, DNA, RNA, carbohydrates, lipids, and other cellular

components; (3) muscle contraction; and (4) secretory and absorptive processes (Guernsey and Edelman, 1983).

The rate of turnover of ^{32}P and its incorporation into ATP in the intact animal were greatly increased by hyperthyroidism (Venkataraman et al., 1950). When a hyperthyroid dog was infused intravenously with inorganic phosphate (Pi), the animal died with hyperthermia indicating that the Pi concentration may be the limiting factor in the rate of oxygen uptake (Roberts et al., 1966).

The increased ATP concentration was probably facilitated by an alteration in the mitochondrial membrane. In hyperthyriodism, the capacity for adenosine diphosphate (ADP) transport from cytosol into the mitochondria was increased (Babior et al., 1973) and the protonic electrochemical potential difference (p) needed for ATP synthesis was lowered, indicating an alteration of the mitochondrial membrane (Shears and Bronk, 1979). In addition, the number of mitochondria per g of tissue was increased in the muscle (Gustafsson et al., 1965; van Hardeveld et al, 1976) but unchanged in the liver of hyperthyroid rats (Jakovcic et al., 1978).

In conclusion, an increased concentration of thyroid hormones appears to cause an increased ATP utilization rather than mitochondrial uncoupling.

Increased gluconeogenesis. In hyperthyroidism, there is an increased activity of the following enzymes involved

in gluconeogenesis: (1) pyruvate carboxylase; (2) phosphoenolpyruvate carboxykinase (Bottger et al., 1970; Muller et al., 1982) and (3) glucose 6-phosphatase (Szepesi and Freedland, 1969); however, the activity of hexose diphosphatase is actually decreased in the livers of hyperthyroid rats (Murad and Freedland, 1967). Because this enzyme is the allosteric enzyme which regulates glucose biosynthesis (Leninger, 1975), gluconeogenesis is probably not greatly increased in hyperthyroidism.

Futile fat cycle. The activities of acetyl coenzyme A carboxylase and fatty acid synthetase, two important enzymes of lipogenesis, were increased in the liver and adipose tissue of hyperthyroid rats (Diamant et al., 1972; Kumar et al., 1977). The rate of fatty acid synthesis was also increased in the liver and adipose tissue of hyperthyroid animals (Sprites et al., 1953; Dayton et al., 1960; Gompertz and Greenbaum, 1966; Diamant et al., 1972; Correze et al., 1977). Triglyceride synthesis (Bressler and Wittels, 1966; Fisher and Ball, 1967; Kako and Liu, 1974) and plasma concentrations of fatty acids (Wahren et al., 1978) and triglycerides (Coates et al., 1979) were also increased in hyperthyroid animals. Lipolysis and triglyceride synthesis may be increased simultaneously, forming an energy wasting (futile) cycle which may partially account for the increased oxygen uptake (Bartels and Sestoft, 1980).

Increased Na^+/K^+ATPase activity. The active transport of Na^+ increased in the liver and skeletal muscle of rats treated with small doses of T_3. In liver slices from hyperthyroid rats, inhibition of Na/K-ATPase by ouabain caused a fall in the rate of oxygen uptake to a level almost equal to euthyroid animals (Ismail-Beigi and Edelman, 1970; 1971). The maximum velocity constant (Vm) increased but there was no change in the Michaelis-Menton constant (Km). Because of the magnitude of the increase, this mechanism alone could account for 90% of the increased rate of oxygen uptake (Lo and Edelman, 1976; Asano et al., 1976; Philipson and Edelman, 1977); however, this estimation has been challenged by Folke and Sestoft (1977) who believe it to be an overestimation caused by the use of liver slices. When an isolated perfused liver apparatus was used to estimate the amount of ATP consumed by Na/K-ATPase of euthyroid and hyperthyroid livers, the stimulated activity of Na/K-ATPase could only account for 5% of the total increased energy expenditure.

NADH shuttle. Thyroid hormones may regulate calorigenesis by increasing the mitochondrial use of the α-glycerol phosphate shuttle, while decreasing the use of the malate-aspartate shuttle. In most animal tissues, the mitochondrial membrane is impermeable to NADH; however, the electrons derived from cytosolic NADH can enter the mitochondria via one of these two shuttles. Use of the α-glycerol phosphate shuttle results in 2 moles of ATP per

mole of cytosolic NADH, while the use of the malate-aspartate shuttle results in 3 moles of ATP per mole of NADH (Leninger, 1975). Hyperthyroidism resulted in the induction of enzymes involved in the α-glycerol phosphate shuttle system, indicating a transfer by the mitochondria from the most efficient shuttle system to one which "wastes" energy (Lee et al., 1959; Qellinger et al., 1966; Tishler and Hammond, 1975; Oppenheimer et al. 1977; Tyzbir et al., 1981).

Heartwork. One of the most striking symptoms of hyperthyroidism is the hyperactivity of the cardiovascular system (see DeGroot and Niepomniszcze, 1977). In hyperthyroid animals, cardiac output, heart rate, and systolic blood pressure were increased with no change in the diastolic (see Tipperman, 1980). In human hyperthyroidism, the basal metabolism rate (BMR) of the heart was found to be increased by 50% and the heart rate by 80% (Amidi et al., 1968). In hyperthyroidism, there was an increased speed of shortening of the contractible elements and an increased rate of tension development (Buccino et al., 1967). Although the mechanism was unknown, it possibly involved an increased activity of myosin (Morkin et al., 1977).

The increase in the rate of oxygen uptake in hyperthyroidism may be partially accounted for by the increased activity of the heart. The rate of oxygen consumption in hyperthyroidism was proportionally related to the heart rate

(Rowe et al., 1956). In euthyroid individuals, the heart accounts for 15-20% of the BMR. In hyperthyroid subjects, it may account for 30-40% (Sestoft, 1980).

Halogenated Chemicals Affecting Thyroid Function

Because there are so many chemicals which affect thyroid hormone function, only those containing halogen will be reviewed. For information on other chemicals affecting thyroid function see Cavalieri and Pitt-Rivers (1981) and Haynes and Murad (1980).

Ethyl p-chlorophenoxyisobutyrate (Clofibrate). Clofibrate, used in the treatment of hyperlipidemia, altered the distribution (Osorio et al., 1965), increased the biliary excretion, and decreased serum concentrations of T_4 in rats (Harland and Orr, 1975).

2,2-bis(chlorophenyl-4-chlorophenyl)-1,1-dichloroethane (o,p`-DDD). This drug, used to treat adrenal carcinoma, competed with T_4 for binding to TBG (Marshall and Tompkins, 1968) and reduced the serum protein-bound iodine (PBI), an indicator of serum thyroid hormone level (Danowski et al., 1964). Marshall and Tompkins (1968) pointed out the structural similarities between o,p`-DDD and T_4.

Dichloro-diphenyl-trichloroethane (DDT). DDT given to rats for 4 days increased bile flow and biliary clearance of ^{125}I-T_4 and decreased plasma T_4 concentrations (Bastomsky,

1974). A single large dose of DDT depressed the uptake of iodide by the thyroid gland and the ability of the thyroid gland to concentrate iodide.

Polychlorinated biphenyls (PCB). When fed to rats, PCB increased the following: (1) biliary excretion of labelled T_4, (2) bile to plasma T_4 ratio, (3) bile flow, decreased the following: (1) PBI, (2) plasma protein binding of T_4 (Bastomsky, 1974; Bastomsky and Murthy, 1976) and (3) the peripheral conversion of T_4 to T_3 (Barsano, 1981).

2,4-Dichlorophenoxy acetic acid (2,4-D). Rats treated with 2,4-D followed by a tracer injection of labelled T_4 had lower serum PBI, increased apparent volume of distribution of T_4, and increased proportion of labelled T_4 located in the liver. 2,4-D was also found to be a weak competitor for T_4-binding sites on serum proteins (Florsheim et al., 1963).

Halogenated phenols. Halogenated phenols (2,4; 2,6; and 3,5-dichlorophenol, 2,4,6-triiodophenol and 2,4,6-tribromophenol) competed for T_4 binding sites of serum albumin (Tabachnick et al., 1970). 2,2,6-; 2,4,5-; 2,3,4-; 2,4,6-; 3,4,5-; and 2,3,5-trichlorophenol, 4-bromo-2,5-dichlorophenol, and 3,5,6-trichloro-2-pyridinol also competed with T_4 for serum albumen binding, but 2,6-dimethyl-4-pyridinol and 2,4,5-trichlorophenol were not competitive with T_4 for binding to the mitochondria in rats. Ingestion of 4-bromo-2,5-dichlorophenol and pentachlorophenol by rats signifi-

cantly reduced serum thyroid hormone levels, and ingestion of pentachlorophenol and 3,5,6-trichloropyridinol enlarged the thyroid glands in a dose-related manner (Beck, 1976).

Feed Efficiency

Feed efficiency can be defined as the total weight gained divided by total feed consumed. Agricultural scientists are continuously investigating methods of increasing feed efficiency because increasing feed efficiency would increase the profit margin of the farmer and the yield of agricultural goods. Halogenated phenol-like compounds are under investigation for their ability to increase feed efficiency.

Dairy cows fed corn silage containing residues of leptophos had increased body gains and decreased milk production (Table 2), but feed intake was not affected (Johnson et al., 1971). The major metabolite of leptophos is 4-bromo-2,5-dichlorophenol. In rats, 12% of the administered dose of leptophos was excreted in the urine as this metabolite. It was also the major metabolite found in cows (Johnson et al. 1971), cotton plants (Holmstead et al., 1973) and houseflies (Lee and Fukuto, 1976).

Ronnel, another organophosphate insecticide, increased feed efficiency in cattle (Rumsey et al., 1975; Riley and Ware, 1977; Wooden and Algeo, 1977; Thomas and Ware, 1978; Rumsey, 1979; Rumsey et al., 1981) and rats (Trankina et

al., 1979; 1982; Trankina et al., 1982). Ronnel was also found to increase serum T_4 levels but not T_3 (Bitman and Rumsey, 1980). In rats, 41 to 47% of the administered dose of ronnel was excreted in the urine as 2,4,5-trichlorophenol (Bradway et al., 1977).

Unmetabolized leptophos and ronnel may be the species responsible for the increased feed efficiency, or it may be their phenolic metabolites.

Rats which ingested feed containing 2,4,5-trichlorophenol had greater body weights and weight gains than controls; however, these were not statistically significant (McCollister et al., 1961). Feeding rats high concentrations of 3,5,6-trichloro-2-pyridinol, 2,5,-dimethyl-4-pyridinol, or thiourea resulted in a decreased feed efficiency. Pentachlorophenol resulted in a biphasic phenomenon, i.e. low doses (100 ppm and 200 ppm) resulted in a slight increase and high doses (400 ppm) a significant decrease. Neither 2,4,5-trichlorophenol nor 2,4,6-trichlorophenol had an effect on feed efficiency (Beck 1976).

From unpublished studies performed at Dow Chemical, it appears that trichloropyridinol increased the feed efficiency in a number of different livestock species (Hymus, T., personal communication). These halogenated phenol-like compounds may be increasing feed efficiency by altering normal thyroid function. Decreasing thyroid activity will reduce basal metabolism rate (BMR) accelerating body weight

gains. As an example, cattle with partially removed thyroid glands had better rates of gain than controls (Bullard and Andrews, 1943).

Uses and Persistance of Halogenated Phenols and Pyridinols

In 1841, the first halogenated phenol (pentachlorophenol) was synthesized (Erdmann, 1841; Laurent, 1841). By the 1930's, halogenated phenols were being produced on a commercial scale to be used as a wood preservative (Carswell and Nason, 1938). Presently these compounds are used as fungicides, molluscicides, herbicides and intermediates in the formation of pesticide (McCollister et al., 1959; Bevenue and Beckman, 1967; Roushdy et al. 1974; Yamamoto and Kasuga, 1976, 1977; Imamura et al., 1978).

Trace amounts of isomers of the following halogenated phenols were found in various rivers in the United States: chlorophenol, methyl-chlorophenol, dimethyl-chlorophenol, dichlorophenol, methyldichlorophenol, trichlorophenol, bromodicholrophenol, dimethyldibromophenol, and tribromophenol. Some of these may have originated from water chlorination (Bean et al., 1980a; 1980b). The only use of 2,5,6-trichloropyridinol, that I am aware of, is in the synthesis of Dursban and Dowco 214.

Dursban residues are relatively stable. Corn plants that had been treated with Dursban still had high Dursban residue levels even after 30 days (Leuck et al., 1970).

Radishes, carrots and treated soil all had relatively high Dursban and trichloropyridinol residue levels even after 1 year (Chapman and Harris, 1980).

Objectives

The objectives of this study were to determine if: (1) the ingestion of trichloropyridinol-treated feed would reduce serum thyroid hormone levels and/or reduce the hepatic nuclear binding capacity for T_3 and (2) trichloropyridinol and other related halogenated phenols could compete with T_3 for the hepatic nuclear receptor.

METHODS

Competitve Binding studies.

The 3,5,6-trichloro-2-pyridinol (Dow Chemical Co., Inc., Midland, MI), 2,4,5-trichlorophenol (Dow Chemical Co.), 4-bromo-2,5-dichlorophenol (Velsicol Chemical Corporation, Chicago, IL), and 4-iodo-2,5-dichloro-phenol (Agricultural Division of CIBA-GEIGY Corporation, Greensboro, NC) which had purities greater than 94%, were dissolved in 50% ethanol and NaOH, then diluted with pH 7.0 medium containing 0.32 M sucrose, 3 mM $MgCl_2$, 20 mM tris buffer (Sigma Chemical Co., St. Louis, MO), and 0.03% human serum albumin (Sigma Chemical Co.). The final concentrations of ethanol and base used in the incubation were less than 0.1%. Competitive binding assays were performed using the procedure of Koerner et al. (1975). Hepatic nuclei (300 mg protein) from male Wistar rats were prepared and incubated in the incubation medium containing 0.1 pmol ^{125}I-T_3 (New England Nuclear, Boston, MA), 0.32 M sucrose, 3 mM $MgCl_2$, 20 mM tris buffer, 0.03% human serum albumin and increasing concentrations of test chemicals. Non-specifically bound T_3 was also measured and corrected for. The incubation took place at 37^{o} C for 40 minutes in a Dubnoff metabolic shaking incubator oscillating at 100 times per minute. The incubation was stopped by placing the tubes in an ice water bath.

Most of the unbound T_3 was removed by adding 1 ml of 1% Triton X-100 in 0.32 M sucrose. The samples were then kept at 0° C for 15 minutes. After centrifuging at 10,000 X g for 10 min, the pellet was washed with a solution consisting of 0.5% Triton X-100 0.32 M sucrose. The radioactivity of the pellet was measured.

Animal Feeding and Sacrifice.

Wistar rats weighing approximately 100 g were housed individually and given water ad libitum. After an adaptation period, rats were randomly divided into 6 groups and fed diets containing 5, 50, and 500 ppm 3,5,6-trichloro-2-pyridinol, as the sodium salt, or non-treated feed for the first 30 days followed by feed containing 200 and 2000 ppm 2-thiouracil (Sigma Chemical Co.) for the remaining 30 or 60 days. Animals were observed daily and weighed weekly. A set amount of fresh feed was added 3 times per week. Feed remaining at the end of the week was weight and disposed of. After 30, 60, or 90 days, blood was sampled by cardiac puncture, and the rats were sacrificed. Ten rats per group were sacrificied at 30 or 60 days and 20 rats per group at 90 days. Livers were immediately removed and weighed. The main lobe was frozen in liquid nitrogen, and the remaining liver was placed in a 10% formalin solution. Kidneys, spleens, and testes were also weighed and placed in formalin. Thyroid glands were re-

moved, placed in formalin, and weighed at a later date. Adrenyl glands and pancreas were also removed and placed in formalin. Microscopic examinations were made of the organs taken from rats treated with trichloropyridinol.

Feed Efficiency.

Feed efficiency was calculated by dividing the total feed consumed by the total weight gain.

Serum T_4, T_3, and rT_3.

Serum levels were determined by radioimmunoassay (RIA) using kits obtained from Abbott Laboratories (North Chicago, IL) for T_4 and T_3 assays and from Serono Labs (Braintree, MA) for the rT_3 assay.

Serum was incubated with rabbit antiserum and radiolabelled T_4, T_3, or rT_3. After the incubation, polyethylene glycol 800 was added. The pellet from a 1000 X g, 15 min centrifugation was counted for radioactivity. Serum concentrations were found by plotting a standard curve. All standards and samples were performed in duplicate.

T_3 Nuclear Receptor Assay.

On the day of analysis, frozen livers were weighed and pulverized. A solution of 0.34 M sucrose, 15 mM $MgCl_2$, and 0.24 M spermine (Sigma Chemical Co.) at 37° C was added to thaw the livers (Latham et al. 1976). The hepatic nuclei were isolated using the method of Widnell and Tata (1964).

The nuclear T_3 binding capacity was measured using the method of Koerner et al. (1975). Nuclear preparations (300 mg protein) were incubated in 1 ml of medium containing a total of 100 nM T_3, a sufficient concentration to saturate all specific T_3 binding sites. Incubations were conducted with T_3 in varied ratios of ^{125}I-T_3 to non-radiolabelled T_3 (Sigma Chemical Co.). After a 40 min incubation, the tubes were treated as previously described. The radioactivity was measured and plotted against the ratio of labelled:unlabelled T_3. The slope of this line is an estimation of the binding capacity.

Protein Analysis.

Protein content of the nuclear preparation was analyzed by the biuret method (Shatkin, 1969).

Statistical Procedures.

Slopes of lines were obtained by simple linear regression analysis. Analyses of variance were performed on all data. When significant differences ($p < 0.05$) were indicated, data from the test groups were compared to control using Fisher's Least Significant Difference (LSD) to pinpoint groups which were significantly different.

RESULTS

Affinity of Trichloropyridinol and Analogs for T_3 Receptor.

Trichloropyridinol was weakly competitive with T_3 for specific binding sites when isolated hepatic nuclei from untreated rats were incubated with ^{125}I-T_3 and increasing concentrations of trichloropyridinol or halogenated phenols (Table 2). Iododichlorophenol was the most active with an affinity 5.79×10^{-6} that of T_3, followed by bromodichlorophenol, trichlorophenol and trichloropyridinol with affinity ratios of 1.33×10^{-6}, 1.22×10^{-6}, and 0.35×10^{-6}, respectively.

Serum Thyroid Hormone Levels.

Rats ingesting diets containing 0, 5, 50 and 500 ppm trichloropyridinol for 30, 60, and 90 days had lower serum levels of T_4 (Table 3). After 30 days, serum T_4 concentrations were significantly reduced to 5.95 and 4.18 ug/dl in rats ingesting 5 and 500 ppm, respectively, compared to 8.89 ug/dl in rats ingesting a control diet. Those rats that ingested 50 ppm for 30 days also had lower serum T_4 levels (7.64 ug/dl); however, this was not significant ($p<0.05$). After 60 days, serum T_4 concentrations were also reduced in treated animals; however, this was also not significant

TABLE 2

RELATIVE AFFINITIES OF 3,5,6-TRICHLORO-2-PYRIDINOL AND HALOGENATED PHENOLS FOR SPECIFIC HEPATIC NUCLEAR T_3 RECEPTOR SITES

Test Chemical	$(Kdis/KT_3)^a$ X 10
3,5,6-Trichloropyridinol	0.35
2,4,5-Trichlorophenol	1.22
4-Bromo-2,5-dichlorophenol	1.33
4-Iodo-2,5-dichlorophenol	5.79

[a]Ratio of concentration of test chemical required for 50% depression of tracer T binding to the corresponding concentration of non-radiolabelled T_3 required for 50% depression.

TABLE 3

THYROID HORMONE CONCENTRATIONS IN SERA OF RATS INGESTING FEED TREATED WITH 3,5,6-TRICHLORO-2-PYRIDINOL[a]

Exposure Concentration (ppm)	T_4 ug/dl	T_3 ng/ml	rT_3 ng/ml
		30 days	
0	8.89±4.43	1.00±0.094	0.375±0.481
5	5.95±0.99[b]	1.13±0.340	0.042±0.019
50	7.64±0.94	1.20±0.298	0.255±0.580
500	4.18±0.85[b]	0.90±0.105	0.064±0.021
		60 days	
0	7.09±1.99	1.49±1.203	0.115±0.060
5	5.75±2.13	1.13±0.340	0.190±0.247
50	5.59±1.53	1.02±0.148	0.135±0.115
500	5.16±1.77	0.98±0.123	0.098±0.022
		90 days	
0	7.43±1.43	0.735±0.198	0.178±0.192
5	7.02±1.51	0.620±0.248	0.286±0.227
50	6.83±1.96	0.580±0.246	0.065±0.056[b]
500	4.00±0.72[b]	0.770±0.306	0.102±0.195

[a] Mean±SD. Samples from each rat was done in duplicates. There were 10,10, and 20 rats per group at 30, 60, and 90 days.
[b] Significantly different from controls ($p < 0.05$).

($p<0.05$). After 90 days, rats ingesting 500 ppm had significantly lower serum T_4 levels (4.00 ug/dl) than controls (7.43 ug/dl).

The reduction in T_4 was found to be linearly correlated to dose at all three time intervals ($p<0.05$). Serum T_3 and rT_3 levels were generally not significantly affected.

Rats ingesting diets containing 200 ppm and 2000 ppm thiouracil also had serum T_4 levels significantly lower than controls (Table 4). At the 60 day sacrifice, T_4 levels were reduced from 7.09 ug/dl in controls to 4.59 ug/dl in the 200 ppm treatment group and 0.60 ug/dl in the 2000 ppm group. At the 90 day sacrifice, T_4 levels were reduced from 7.43 ug/kl to 4.78 ug/dl and 0.77 ug/dl in the 200 and 2000 ppm groups. Serum T_4 levels of rats fed 500 ppm trichloropyridinol were reduced about the same extent as rats fed 200 ppm thiouracil, a potent inhibitor of thyroid hormone synthesis. The serum T_3 levels were not significantly reduced at the 90 day sacrifice time but were reduced in a dose-dependent manner at the 60 day from 1.4 ng/ml in controls to 0.770 ng/ml in 200 ppm and 0.500 in the 2000 ppm. Serum rT_3 levels were not significantly reduced at the 60 day, but were reduced in a dose-dependent manner at the 90 day from 0.178 ng/ml in controls to 0.125 ng/ml in 200 ppm and 0.34 in the 2000 ppm.

TABLE 4

THYROID HORMONE CONCENTRATIONS IN SERA OF RATS INGESTING FEED TREATED WITH 2-THIOURACIL[a]

Exposure concentration (ppm)	T_4 ug/dl	T_3 ng/ml	rT_3 ng/ml
		60 days	
0	7.09±1.99	1.49±1.203	0.115±0.060
200	4.59±1.34[b]	0.770±0.149[b]	0.380±0.296
2000	0.60±0.40[b]	0.500±0.115[b]	0.212±0.384
		90 days	
0	7.43±1.43	0.735±0.198	0.178±0.192
200	4.78±1.54[b]	0.805±0.182	0.125±0.085
2000	0.77±0.31[b]	0.670±0.236	0.034±0.011[b]

[a]Mean±SD. There were 10 animals per group at 60 days and 20 per group at 90 days.
[b]Significantly different from controls ($p \leq 0.05$).

Nuclear Binding Capacity.

Although there was a slight increase in the nuclear T_3 binding capacity from 208±98 fmole/mg protein in controls to 342±94 in rats ingesting 500 ppm trichloropyridinol for 30 days, this was not significant (Table 5). At 90 days, there was no difference in the binding capacity between controls and treated. No correlation was found between serum T_4 levels and nuclear T_3 binding capacity.

Feed Efficiency, Body Weight, and Organ Weights.

Ingestion of trichloropyridinol did not significantly alter total body weight (Table 6) or feed efficiency (Table 7) in rats. Rats that ingested 2000 ppm thiouracil had significantly lower body weights 302±30 g after 60 days compared to 345±32 g for controls and after 90 days 286±21 g compared to 398±38 g for controls (Table 6). Feed efficiency was also significantly reduced in rats ingesting feed containing 2000 ppm thiouracil. From day 30 to day 60 control rats had a feed efficiency of 0.100±0.026 g/g but those ingesting feed containing the highest concentration of thiouracil had a feed efficiency of only 0.004±0.025 g/g. The difference in feed efficiency of the control rats vs. those fed 2000 ppm thiouracil was almost as striking from day 60 to day 90. Control rats had a feed efficiency of 0.051±0.016 g/g, but rats ingesting 2000 ppm thiouracil had

TABLE 5

T3BINDING CAPACITY OF HEPATIC NUCLEI FROM RATS FED DIETS CONTAINING VARIOUS CONCENTRATIONS OF TRICHLOROPYRIDINOL[a]

Test chemical	Dietary concentration (ppm)	30 days	90 days
Control		208±98	255±88
Trichloropyridinol	5	293±95	195±94
	50	283±89	238±94
	500	342±94	204±57

[a]Results are mean±SD fmole T3/mg protein. There were 6 animals in each group in the 30 day trial and 5 in the 90 day.

TABLE 6

TOTAL BODY WEIGHTS OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL[a]

Treatment group (ppm)	Total body weight (g) 0 Day	30 Days	60 Days	90 Days
Control	120±11[b]	145±12[b]	345±32[c]	398±38[d]
Pyridinol, 5	121±11	151±28	360±25	409±28
Pyridinol, 50	119±11	151±19	348±25	399±31
Pyridinol,500	121±9	137±37	341±59	400±36
Thiouracil, 200	119±15	-----	340±35	394±31
Thiouracil, 2000	118±15	-----	302±30	286±21[e]

[a]Mean±SD.
[b]Significantly different from controls ($p \leq 0.05$).
[c]n=40
[d]n=30
[e]n=20

TABLE 7

FEED EFFICIENCY OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL

Treatment Group(ppm)	30 Days	60 Days	90 Days
	Weight gained/Feed consumed(g/g)[a]		
Control	0.277±0.047[c]	0.193±0.018[d]	0.155±0.011[e]
Pyridinol, 5	0.301±0.026	0.209±0.019	0.162±0.016
Pyridinol, 50	0.285±0.030	0.193±0.022	0.156±0.011
Pyridinol, 500	0.288±0.053	0.187±0.015	0.154±0.011
Thiouracil, 200	-----	0.185±0.028	0.154±0.018
Thiouracil, 2000	-----	0.157±0.024	0.118±0.013[b]
	Weight gained/Feed consumed in the last 30 days(g/g)		
Control	-----	0.100±0.026	0.051±0.016
Pyridinol, 5	-----	0.110±0.024	0.051±0.022
Pyridinol, 50	-----	0.110±0.058	0.057±0.022
Pyridinol, 500	-----	0.093±0.022	0.048±0.015
Thiouracil, 200	-----	0.090±0.033	0.043±0.023
Thiouracil, 2000	-----	0.004±0.025[b]	0.014±0.037[b]

[a] Mean±SD.
[b] Significantly different from controls ($p \leq 0.05$).
[c] n=40
[d] n=30
[e] n=20

TABLE 8

LIVER WEIGHTS OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL[a]

Treatment (ppm)	30day	60day	90day
		Liver wt. (g)	
Control	10.56±1.00	12.14±1.02	12.50±1.70
Pyridinol, 5	11.48±1.12	12.87±1.91	12.84±1.21
Pyridinol, 50	11.37±0.87	11.67±1.40	12.23±2.13
Pyridinol, 500	10.64±1.67	12.81±0.99	12.62±1.71
Thiouracil, 200	----	12.43±1.51	12.98±1.72
Thiouracil, 2000	----	11.38±1.37	9.36±0.95[b]
		Liver wt/body wt (g/kg)	
Control	39.6±2.7	35.9±2.0	31.4±3.3
Pyridinol, 5	42.4±2.2	35.2±3.6	31.4±2.8
Pyridinol, 50	40.6±2.3	34.0±4.4	31.5±4.4
Pyridinol, 500	40.7±3.0	36.0±1.9	31.5±2.8
Thiouracil, 200	----	36.3±1.7	33.0±4.3
Thiouracil, 2000	----	37.2±3.0	32.7±2.5

[a]Mean±SD. There were 10, 10, or 20 animals per group of the 30, 60, or 90 day sacrifice periods, respectively.
[b]Significantly different from controls ($p \leq 0.05$).

TABLE 9

KIDNEY WEIGHTS OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL[a]

Treatment (ppm)	30day	60day	90day
		Kidney wt. (g)	
Control	2.09±0.22	2.44±0.27	2.45±0.30
Pyridinol, 5	2.04±0.37	2.87±0.21	2.58±0.18
Pyridinol, 50	2.19±0.15	2.23±0.22	2.47±0.23
Pyridinol, 500	2.06±0.22	2.64±0.21	2.46±0.24
Thiouracil, 200	----	2.36±0.22	2.46±0.36
Thiouracil, 2000	----	1.82±0.23[b]	1.77±0.16[b]
		Kidney wt/body wt (g/kg)	
Control	7.79±0.67	7.12±0.42	6.14±0.38
Pyridinol, 5	7.96±0.52	6.61±0.35	6.32±0.63
Pyridinol, 50	7.73±0.50	6.50±0.63	6.21±0.39
Pyridinol, 500	7.91±0.70	7.41±0.43	6.18±0.45
Thiouracil, 200	----	6.88±0.58	6.25±0.79
Thiouracil, 2000	----	6.20±0.45[b]	6.19±0.43

[a]Mean±SD. There were 10, 10, or 20 animals per group at the 30, 60, or 90 day sacrifice periods, respectively.
[b]Significantly different from controls ($p \leq 0.05$).

TABLE 10

SPLEEN WEIGHTS OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL[a]

Treatment (ppm)	30day	60day	90day
		spleen wt. (g)	
Control	0.56±0.08	0.64±0.06	0.68±0.09
Pyridinol, 5	0.57±0.08	0.65±0.09	0.69±0.07
Pyridinol, 50	0.58±0.08	0.67±0.06	0.67±0.06
Pyridinol, 500	0.53±0.10	0.63±0.09	0.64±0.10
Thiouracil, 200	----	0.63±0.08	0.64±0.09
Thiouracil, 2000	----	0.37±0.06[b]	0.32±0.05[b]
		spleen wt/body wt (mg/kg)	
Control	2.1±0.3	1.9±0.2	1.7±0.2
Pyridinol, 5	2.1±0.2	1.8±0.2	1.7±0.2
Pyridinol, 50	2.1±0.2	2.0±0.2	1.7±0.1
Pyridinol, 500	2.0±0.3	1.8±0.2	1.6±0.2
Thiouracil, 200	----	1.8±0.2	1.6±0.2
Thiouracil, 2000	----	1.3±0.1[b]	1.1±0.1[b]

[a]Mean±SD. There were 10, 10, or 20 animals per group at the 30, 60, or 90 day sacrifice periods, respectively.
[b]Significantly different from controls ($p \leq 0.05$).

a feed efficiency of 0.014±0.037 g/g. The reduction in feed efficiency and weight gains may have been caused by the unpalatability of the feed.

Liver, spleen, and kidney weights were significantly less in animals fed 2000 ppm thiouracil (Tables 8, 9 and 10) but not if the reduction in body weight was corrected for - except those sacrificed on the 60th and 90th day of the study in which the corrected kidney weights and spleen weights were still reduced. The weights of the testes were about the same as controls in all treatment groups; however, the weights of testes compared to body weight were significantly increased in the 2000 ppm thiouracil group (Table 11). Liver, spleen, kidney, testes, adrenyl, thyroid gland, and pancreas were examined by a qualified pathologist and found to be normal in all the animals treated with trichloropyridinol. Organs from rats ingesting thiouracil were not examined histologically.

The weights of the thyroid glands were not affected by trichloropyridinol ingestion but were increased in a dose-related manner in rats fed thiouracil at both sacrifice time periods (Tables 12). Thyroids from rats ingesting thiouracil were 2 to 5 times the size of controls.

TABLE 11

TESTIS WEIGHTS OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL[a]

Treatment (ppm)	30day	60day	90day
		testis wt. (g)	
Control	2.57±0.17	2.95±0.15	3.07±0.30
Pyridinol, 5	2.65±0.16	2.94±0.25	3.04±0.27
Pyridinol, 50	2.63±0.15	2.93±0.24	3.02±0.32
Pyridinol, 500	2.53±0.16	3.02±0.18	3.06±0.36
Thiouracil, 200	----	2.89±0.31	3.06±0.24
Thiouracil, 2000	----	2.88±0.18	2.99±0.31
		testis wt/body wt (g/kg)	
Control	9.62±0.80	8.65±0.57	7.75±0.79
Pyridinol, 5	9.83±0.87	8.10±0.92	7.46±0.64
Pyridinol, 50	9.38±0.71	8.53±0.68	7.59±0.69
Pyridinol, 500	9.79±1.20	8.51±0.62	7.69±0.91
Thiouracil, 200	----	8.47±0.82	7.80±0.73
Thiouracil, 2000	----	9.46±0.42	10.47±0.87[b]

[a] Mean±SD. There were 10, 10, or 20 animals per group at the 30, 60, or 90 day sacrifice periods, respectively.
[b] Significantly different from controls.

TABLE 12

THYROID WEIGHTS OF RATS INGESTING 3,5,6-TRICHLORO-2-PYRIDINOL OR 2-THIOURACIL[a]

Treatment (ppm)	30day	60day	90day
		thyroid wt. (mg)	
Control	11.3±7.1	13.0±3.0	18.8±4.2
Pyridinol, 5	13.0±2.0	14.0±2.1	19.7±3.9
Pyridinol, 50	15.7±2.8	13.0±3.1	18.5±2.8
Pyridinol, 500	12.0±2.2	15.0±6.2	18.3±3.9
Thiouracil, 200	----	21.1±4.9[b]	31.6±6.9[b]
Thiouracil, 2000	----	49.1±8.0[b]	80.5±19.6[b]
		thyroid wt/body wt (ug/kg)	
Control	43.0±26.4	38.1±8.0	47.2±10.2
Pyridinol, 5	47.3±9.3	38.3±4.6	48.5±11.1
Pyridinol, 50	56.1±10.2	38.1±10.1	46.1±6.8
Pyridinol, 500	46.6±8.1	42.3±17.5	45.8±9.3
Thiouracil, 200	----	61.0±8.5[b]	80.3±15.1[b]
Thiouracil, 2000	----	169.6±3.4[b]	280.8±65.1[b]

[a]Mean±SD. There were 10, 10, or 20 animals per group at the 30, 60, or 90 day sacrifice periods, respectively. Weights of the thyroid glands were made after formalin fixation.
[b]Significantly different from controls ($p \leq 0.05$).

DISCUSSION

Trichloropyridinol affects various facets of normal thyroid hormone function. Trichloropyridinol and trihalophenol analogs competed with T_3 *in vitro* for specific nuclear receptor sites. Although this competition was weak, it may be important in the intact animal provided the tissue concentration of the halogenated compound is sufficiently large compared to T_3.

Trichloropyridinol ingestion did not affect the number of free sites available for T_3 binding in hepatic nuclei under the conditions of the experiment. These binding sites consist mainly of specific T_3 receptors with some lower affinity non-specific sites. However, the use of Triton X-100 removed most of the non-specifically bound T_3; therefore, most of the bound T_3 probably was bound to high affinity specific T_3 receptors.

Binding capacities of 204 to 342 fmole T_3/mg protein found in these nuclei were comparable to binding capacities of 363 to 836 fmole/mg protein found in chick embroys (Bellabarba and Lehoux, 1983) and 253 to 520 fmole/mg protein found in human peripheral lymphocytes (Wartofsky *et al.*, 1981).

The discrepancy between the findings that the number of

free sites available for T_3 binding in the hepatic nuclei of rats chronically fed trichloropyridinol and the finding that trichloropyridinol competes with T_3 for nuclear receptors can be explained two ways: (1) the concentration of trichloropyridinol relative to T_3 was not sufficient in those rats ingesting trichloropyridinol to permit measurement of the reduction of available receptor sites and (2) trichloropyridinol bound to nuclear receptors so weakly during the measurement that it dissociated from the receptors in the hepatic nuclei.

Serum T_4 levels were found to be reduced in rats ingesting trichloropyridinol to levels similar to those found with thiouracil, an inhibitor of thyroid hormone synthesis; however, thiouracil resulted in a reduction in the levels of T_3 and rT_3, but trichloropyridinol did not. Aizawa and Yamada (1981) found a plasma T_4 concentration of 4.4 ug/dl in normal control rats. The level was reduced to 0.3 ug/dl in thyrodectomized rats. We found 7 ug/dl in control rats in this study, but 0.6 ug/dl in rats that had ingested 2000 ppm thiouracil indicating that thyroxine synthesis had been almost completely inhibited. Aizawa and Yamada (1981) found plasma T_3 levels were reduced from 0.97 ng/ml to 0.15 ng/ml when rats were thyrodectomized. Ingestion of 2000 ppm thiouracil caused a reduction in serum T_3 from 1.49 ng/ml to 0.5 ng/ml in this study.

Trichloropyridinol did not alter the weight or the gross anatomy or microscopic appearance of the thyroid glands or any of the other organs examined in this study, but thiouracil enlarged the thyroid gland in a dose-dependent manner. This may indicate that T_4 reduction caused by trichloropyridinol was via a different mechanism. Thiouracil inhibits the iodination of the thyroid hormone precursors probably inhibiting the thyroid peroxidase enzyme (Engler et al., 1982).

Polychlorinated biphenyls (PCB) induced hypothyroidism which was physiologically similar to that found with trichloropyridinol; T_4 levels were reduced while T_3 levels remained unchanged (Bastomsky, 1977). PCB appears to displace T_4 from serum proteins and increase biliary excretion of T_4, both resulting in lower serum T_4 levels. In addition, the peripheral deiodinization of T_4 to T_3 was enhanced explaining the absence of reduction in T_3 levels (Barsano, 1981).

Competitive binding studies indicated that trichloropyridinol may displace T_4 from serum proteins in a manner similar to PCB (Nelson, R. M., personal communication) and may be the mechanism for reduction of serum T_4 levels. Further work is needed to determine the effect of trichloropyridinol on the deiodination enzymes.

Serum T_3 levels were depressed in rats ingesting thiouracil at the 60 day sacrifice but not at the 90 day;

however, the rT_3 pattern was opposite. At the 60 day time point, serum rT_3 was normal. At 90 days, rT_3 was depressed. This indicates that the deiodination of T_4 probably shifted from the formation of the inactive rT_3 to the active T_3 until the serum T_3 was returned to a normal level.

In this study, trichloropyridinol did not affect feed efficiency or weight gain in rats as it did in other animals (Hymus, T., personal communications). This is in agreement with the finding of Beck (1976), who also found that trichloropyridinol did not increase the feed efficiency in rats, indicating that rats may not be sensitive to the effects of trichloropyridinol on feed efficiency. Possibly small changes in serum T_4 are handled more effectively in rats than other animals although there is no indication of this in the literature.

Rats fed 2000 ppm thiouracil had lower weight gains and smaller consumption of feed which may be an indication the feed was unpalatable or may be depression in appetite.

Ronnel significantly increased the feed efficiency in rats from 0.156 g/g/day to 0.151 g/g/day (Trankina et al., 1979) and in cattle from 0.097 to 0.109 (Rumsey et al., 1975), but affected serum thyroid hormone levels differently than trichloropyridinol; serum T_4 increased instead of decreased with ronnel in cattle (Bitman and Rumsey, 1980). Beck (1976) found that 2,4,5-trichlorophenol, the mammalian

metabolite of ronnel, increased serum T_4 levels at 100 ppm and reduced it at 400 ppm.

Leptophos also increased feed efficiency in cattle from 0.035 g/g/day to 0.135 g/g/day (Johnson et al., 1971). Its mammalian metabolite is 4-bromo-2,5-dichlorophenol, which Beck (1976) found decreased serum T_4 levels in rats in a dose-related manner. Rats ingesting 100, 200, or 400 ppm 4-bromo-2,5-dichlorophenol had serum T_4 levels of 7.9, 6.3, and 4.7, respectively, compared to serum T_4 levels of 8.0 in control rats.

Both 2,4,5-trichlorophenol and 4-bromo-2,5-dichlorophenol were found to be as competitive as trichloropyridinol for nuclear T_3 receptors in our study.

In conclusion, the effects of trichloropyridinol upon animal growth and feed efficiency may involve several mechanisms surrounding thyroid hormone expression including reduction of serum levels of T_4 and competing with T_3 for the nuclear receptor.

SUMMARY

At relatively high concentrations, trichloropyridinol, 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol and 4-iodo-2,5-dichlorophenol competed with T_3 for specific nuclear receptors. Previous studies had indicated that trichloropyridinol accumulated in the liver where it may alter some of the functions of the thyroid hormone. Male rats ingesting feed containing 5, 50 or 500 ppm trichloropyridinol for 30, 60, or 90 days had serum T_4 levels that were significantly reduced in a dose-related manner. Trichloropyridinol was as potent as thiouracil in reducing serum T_4 levels, a well known thyroid toxicant. Serum T_3 and rT_3 levels, which were reduced by thiouracil ingestion, were not altered by trichloropyridinol. Trichloropyridinol did not alter the hepatic nuclear T_3 binding capacity. Body weight and organ weights and histology were not significantly altered by chronic ingestion of trichloropyridinol. In addition, in opposition to results found using larger animals, trichloropyridinol did not significantly increase feed efficiency in rats.

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APPENDIX

TABLE 13

The ability of 3,5,6-trichloropyridinol, 2,4,5-trichlorophenol, 4-bromo-2,5-dichlorophenol and 4-iodo-2,5-dichlorophenol to displace ^{125}I -triiodothyronine from specific receptors in isolated hepatic nuclei[a]

Test chemical Concentrations	Trichloro-pyridinol	Trichloro-phenol	Bromo-dichlorophenol	Iododi-chlorophenol
10^{-7}	10	16	12	5
10^{-6}	18	17	20	27
10^{-5}	22	20	37	45
10^{-4}	34	39	48	71
10^{-3}	58	70	66	53

[a]Each value is the mean of 3 observations and represents the percent of specifically bound ^{125}I -triiodothyronine displaced by the test chemicals.

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