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TR-6783

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 15 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Denis Edwards, Product Manager #12
Insecticide-Rodenticide Branch
Registration Division (TS-767)

FROM: Roger Gardner, Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769) 7-13-88

THRU: Judith Hauswirth, Ph. D., Head
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769) 7/13/88

SUBJECT: Experimental Use Permit (EPA Reg. No. 45639-EUP-GG) and Tolerances for Residues of APOLLO® on Apples. Meat, and Milk (Petition Nos. 6F3392 and 6H5500) Tox. Chem. No. 593A; Tox. Project Nos. 8-0366, 8-0453, and 8-0572.

Actions Requested

1. Experimental Use Permit for a 50 SC formulation (45639-EUP-GG) on fresh apples.

2. Permanent Tolerances for residues of APOLLO® on fresh apples at 1 ppm (Petition No. 6F3392) and in cattle (fat, meat and meat by products) and milk at 0.01 ppm, kidney at 0.05 ppm, and liver at 0.1 ppm (Petition No. 6H5500).

3. Review of studies listed in Appendix II below.

Recommendations and Conclusions

1a. Acute oral and dermal toxicity studies suggest that technical grade clofentezine should be classified into Toxicity Category III. An acute inhalation toxicity study with an 80 WP formulation suggests that the technical grade material should also be classified into Toxicity Category III for acute inhalation. Results of a primary dermal irritation study indicated that technical grade clofentezine should be classified into Toxicity Category IV, and a study on the 80 WP formulation suggests that technical clofentezine should be classified into Category IV with respect to eye irriration The technical material is also a skin sensitizer.

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1b. Acute oral and dermal toxicity studies on the 50 SC formulation indicate that it should be classified into Toxicity Category IV. Studies with the formulation indicate that it should be placed in Category IV for skin and eye irritation.

2. The lowest no-observed-effect level (NOEL) was established at 50 ppm (1.25 mg/kg/day) in a one-year dog feeding study. Observed effects included elevated serum cholesterol and triglyceride levels in animals given diets containing 1000 or 20,000 ppm. The LEL was established at 1000 ppm (25 mg/kg/day).

3. A chronic feeding study in rats indicated that the NOEL is 40 ppm (2 mg/kg/day), and the LEL is 400 ppm (20 mg/kg/day; highest dose tested). The effects included increased liver weights and liver-to-body weight ratios, increased incidence of centrilobular hepatocyte hypertrophy, and elevated free thyroxine levels in 400 ppm dose group males. These results are consistent with subchronic study results.

4. The incidence of thyroid follicular cell tumors was increased in male rats given clofentezine in the diet at a level of 400 ppm for 27 months. The only tumors that were apparently increased by clofentezine in the diet of mice were liver cell adenomas and carcinoma in females. These results have been considered by the Toxicology Branch Peer Review Committee (see Point 12 below).

5a. Maternal toxicity (slight centrilobular hepatocellular enlargement) was observed in pregnant rats given 3200 mg/kg/day during gestation. The NOEL for that effect was 1280 mg/kg/day. No teratogenic or fetotoxic effects were reported at any of the doses tested, and the NOEL was reported to be the highest dose tested (3200 mg/kg/day). The adult-to developmental toxicity ratio (A:D ratio) in rats is less than one.

5b. In rabbits, slight maternal toxicity (reduced food consumption and body weight gain) as well as slight fetotoxicity (reduced litter and fetal weights) were observed at the 3000 mg/kg/day dose level (highest dose tested). The NOEL for these effects was 1000 mg/kg/day. No compound related increases in developmental effects were observed in the study. The A:D ratio suggested by these results is one.

6. Results of a multigeneration reproduction study suggest that APOLLO® has no reproductive toxicity in rats at dietary levels of 4, 40, or 400 ppm.

7a. Apollo did not induce point mutations in bacteria (Salmonella typhimurium). There were no dominant lethal mutations induced by the chemical in rats, and no induction of micronuclei was observed in treated mice. There were also no gene conversion and mitotic recombination effects in yeast exposed to Apollo.

7b. No point mutations were induced in mammalian cells in vitro (mouse lymphoma cells), but the study did not contain specific historical control data to completely support the conclusions of the investigators.

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8a. Treated rats readily absorbed single oral doses of clofentezine with maximum accumulation of residues in the liver and kidney. Those residues were subsequently cleared within 24 hours after treatment. Almost all of a single oral dose is excreted within 24 to 48 hours after administration regardless of its amount, and the major route of excretion is the feces. Metabolites identified in the urine of treated rats and baboons were free and conjugated hydroxylated derivatives of clofentezine. No fecal metabolites have been identified.

8b. Repeated dosing of rats demonstrated that tissue levels of clofentezine residues reach a plateau after 5 to 15 daily doses. Residue concentrations in the liver and kidneys were 2 to 4 times those reported after a single dose. One study also indicated that the chemical does not readily pass across the placenta in pregnant rats.

9. A dermal absorption study showed that absorption was low (less than 1%) during the 10 hours that followed application of a simulated 50 SC formulation to the skin of rats.

10a. Data gaps include an acute inhalation study or data to indicate that the formulation is not in a respirable form under conditions of normal application, identification of fecal metabolites of clofentezine, and data on historical control cultures from the laboratory where the mouse lymphoma mutation assay was conducted.

10b. A long-term feeding study (>13 weeks in duration) with a broad dose range (>400 ppm) and serial sacrifices is needed to more substantially support the proposed mechanism of thyroid tumor induction observed in the rat chronic feeding study. A protocol for this study should be submitted for approval by the Agency before the study is initiated.

11. Using the NOEL established in the one-year dog study and a Safety Factor of 100, an Acceptible Daily Intake (ADI) of 0.0125 mg/kg/day is derived.

12. Available data have been considered in a weight-of-the-evidence analysis by the Toxicology Branch's Peer Review Committee and the FIFRA Science Advisory Panel, and clofentezine has been classified into Group C (possible human carcinogen). A quantitative risk assessment is considered inappropriate for the following reasons:

    a. The increased tumor incidence was marginally increased above the control incidence ($p = 0.048$) only at the highest dose tested (400 ppm) in the chronic feeding study.

    b. The increased incidence was observed only in male rats.

    c. The thyroid tumor incidence in the chronic feeding study's highest dose group (20%) was slightly greater than the historical range provided by limited data (7.5 to 15%; from 2 studies). (Data were adjusted for animals dying prior to appearance of the first thyroid tumor.)

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d. The additional thyroid function studies suggest the possibility of an indirect mechanism for follicular cell tumor induction that may be associated with clofentezine's liver toxicity.

13. There are adequate Toxicology data to support the requested Experimental Use Permit and proposed tolerances on apples, meat and meat by products of cattle, liver, kidney, and milk provided dietary exposure estimates do not exceed the ADI (see Point 11. above).

I. Background

A. General Information

APOLLO® is proposed for use as a miticide on apples. Its common name is clofentezine, and its chemical name is 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine. The pesticide is formulated for the proposed use as a suspension concentrate (50% active ingredient). The inert ingredients have been cleared for food use.

The formulation, which is called APOLLO MITICIDE (50% SC), is to be applied at the rate of 4 to 8 oz./A according to the label, and the seasonal application rate is not to exceed one pint per acre. The miticide is to be sprayed with ground equipment.

B. Summary of Previously Submitted Data

Appendix I includes the Toxicology Branch "One-Liners" for the studies described in this Section.

1. Acute Toxicity

The acute toxicity categories for technical grade and 50 SC formulations of clofentezine are summarized as follows:

Type of Toxicity	Toxicity Categories: Technical grade	Toxicity Categories: APOLLO® 50 SC
Acute oral	III	IV
Acute dermal	III	IV
Acute inhalation	--*	--**
Primary skin irritation	IV	IV
Primary eye irritation	--†	III

*Based on a study with an 80% WP formulation (see Appendix I below).

**This study is discussed in Section I. C. 1. on page 8 below. Previously, data on the 80 WP formulation were used and suggested a Toxicity Category III for acute inhalation toxicity.

†These data are unavailable on the technical grade material. However, a study on an 80 WP formulation (80% a. i.) suggested a Toxicity Category of IV.

The most frequently observed compound-related effect reported in all but the dermal studies was the discoloration of feces in treated animals. The test substance was described as a magenta colored powder, and the feces of treated animals appeared pink in color for 25 to 30 hours after treatment (84 hours after i. p. injection). At necropsy in an intraperitoneal study, precipitate of the test material was observed in the abdomen of treated rats.

Clofentezine caused dermal sensitization in two of twenty treated female guinea pigs which suggested that the chemical is a weak sensitizer.

2. Subchronic Toxicity

In the 90-day rat feeding study a no-observed-effect level (NOEL) of 40 ppm (2 mg/kg/day) was demonstrated, and the lowest-effect-level (LEL) was reported to be 400 ppm (20 mg/kg/day). Effects noted at that dose level were centrilobular hepatocellular enlargement, elevated mixed-function oxidase enzyme activities and a slight increase in liver weights.

In a 90-day dog study, a NOEL was not established with respect to increased liver weights or changes in electrocardiograms. The lowest dose tested was 3200 ppm (80 mg/kg/day).

Discoloration of the urine and feces by clofentezine was also noted in treated animals in these subchronic studies.

3. Chronic Toxicity

a. Rats

The NOEL in a 27-month feeding study was 40 ppm (2 mg/kg/day), and the LEL was 400 ppm (20 mg/kg/day, highest dose tested). Effects at the 400 ppm dose for male rats included a slight increase in free thyroxine levels in blood sampled at 27 months, increased liver weights and liver-to-body weight ratios, centrilobular hepatocyte hypertrophy and vacuolation, focal cystic degeneration of hepatocytes, and diffuse distribution of fat deposits in the liver. The only changes observed in 400 ppm group females were slightly increased liver weights.

Based on these observations, a no-effect level was established at 40 ppm (2 mg/kg/day), and the lowest-effect level was 400 ppm (20 mg/kg/day).

b. Dogs

In a one-year dog feeding study using levels of 0, 50, 1000, or 20,000 ppm, a NOEL with respect to elevated serum cholesterol and triglyceride levels was established at 50 ppm (reported daily intake ranged from 1.52 to 2.47 mg/kg/day). Electrocardiograms were not affected in dogs given the 20,000, 1000, or 50 ppm diets. Based on these results a NOEL of 50 ppm (1.25 mg/kg/day) and an LEL of 1000 ppm (25 mg/kg/day) is suggested for dogs.

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4. Oncogenicity

a. Rats

In the rat chronic feeding study described above, the incidence of thyroid follicular cell tumors in male rats was statistically significantly increased (see Table 1).

Table 1

The Incidence of Benign and/or Malignant Thyroid Follicular Cell Tumors in Male Rats.

Observation	Dose level (ppm) 0	10	40	400
Type of tumor				
Benign	1/70	1/70	0/70	3/70
Malignant	1/70	1/70	2/70	5/70
Total*	2/70	2/70	2/70	8/70**

*Statistically significant positive dose-related trend ($p<0.05$).
**Statistically significantly different from control group ($p=0.048$: Fisher's Exact Test)

b. Mice

Clofentezine was administered in the diet to mice for up to 105 weeks at levels of 0, 50, 500, or 5000 ppm. Statistically significant reductions in group mean body weight (approximately 7% below control means) and body weight gains (approximately 15 to 22% less than that for control group) were observed in males at 5000 ppm. The highest dose group males showed a statistically significantly increased incidence of altered hepatocytes (areas or foci of eosinophilic hepatocytes). In female mice given the 5000 ppm diet for up to two years, there were statistically significant increases in liver weights and the incidence of altered hepatocytes (eosinophilic and/or basophilic areas or foci).

The incidence of hepatocellular tumors was increased in some test groups, but the increases were not dose-related in either sex.

5. Developmental Toxicity

Maternal toxicity (slight centrilobular hepatocellular enlargement) was observed in pregnant rats given 3200 mg/kg/day (highest dose tested) during gestation. The NOEL for that effect was 1280 mg/kg/day. No teratogenic or fetotoxic effects were reported at any of the doses tested, and the NOEL was reported to be the highest dose tested (3200 mg/kg/day).

In a study with rabbits, slight maternal toxicity (reduced food consumption and body weight gain) as well as slight fetotoxicity (reduced litter and fetal weights) were observed at the 3000 mg/kg/day dose level (highest dose tested). The NOEL for these effects was 1000 mg/kg/day. No compound-related increases in teratogenic effects were observed in the study.

6. Reproduction Toxicity

There were no reproductive effects observed in rats given diets containing 0, 4, 40, or 400 ppm clofentezine. The 400 ppm diet caused centrilobular hepatocyte hypertrophy in the male rats in the study, and the NOEL with respect to that effect was 40 ppm.

7. Mutagenicity

No mutagenic effects were reported in bacterial point mutation assays, gene conversion and mitotic recombination studies in yeast, a mouse micronucleus test, or a dominant lethal study in rats.

Although a dose-related increase in mutation frequency was noted in a mouse lymphoma point mutation assay, the report did not contain specific information to support the conclusions of the investigators.

8. Metabolism

Studies with rats, mice, dogs, rabbits, and baboons demonstrated that the feces was the major route of excretion (70-80% of the administered dose in all species).

Almost all of the administered dose was recovered within 24 to 48 hours after treatment, and in rats the proportion of fecal excretion at high doses (1000 mg/kg) increased to 95-98% of the dose. The plasma half life of clofentezine in rats after administration of 1000 mg/kg was 3.6 hours.

The metabolites identified in the urine of treated rats and baboons were free and conjugated hydroxylated derivatives which accounted for approximately 70-80% of the radiolabel extracted. The feces were not analyzed for identification of residues.

In the rat, intravenous administration resulted in approximately the same proportions of excreted residues in the feces and urine as were noted after oral dosing suggesting excretion in the bile as the major route. Whole body radiography of treated rats also demonstrated the accumulation of residues in the liver and kidney which were subsequently cleared within 24 hours after treatment. Repeated dosing of rats at 20 mg/kg/day demonstrated that tissue levels of clofentezine residues reached a plateau after 5 to 15 daily doses. Residue concentrations in those two organs were 2 to 4 times those reported after a single dose.

The fetuses of pregnant rats treated with 3200 mg/kg/day doses of clofentezine during gestation had residue concentrations which were only one-fifth that of maternal blood or tissues which indicates that the chemical does not readily pass across the placenta.

9. Dermal Absorption

Radiolabeled clofentezine in a 50 SC formulation was applied to the skin of male rats at dose levels of 4.8, 44, or 180 mg per kg body weight, and absorption was low (less than 1%) during the 10 hours that followed application of all doses.

10. Special Studies - Thyroid Effects

Because of the thyroid effects observed in the chronic rat feeding study, a series of studies were conducted, and they indicated the following:

a) There was no preferential accumulation of Apollo in the thyroid.

b) Apollo caused statistically significant treatment-related decreases in the thyroxine half-life in treated rats.

c) There was a significant and rapid increase in thyroid uptake of iodine in Apollo-treated rats.

In a 6-week feeding study, groups of male and female rats were fed diets containing 0, 400, or 30,000 ppm Apollo. Increases in thyroxine, free thyroxine index, thyrotrophin, progesterone, and dehydroepiandrosterone were seen in both sexes at 30,000 ppm. Males given the highest dose also showed an increased level of tri-iodothyroxine. At the 400 ppm dose level, males also had increased thyroxine levels, and females had increased dehydroepiandrosterone levels.

C. Previous Agency Assessments of Clofentezine Toxicity

1. Data Gaps

Technical difficulties with generation of test atmospheres were described as the reason an acute inhalation LC_{50} could not be determined. Efforts to overcome the difficulties did not include dilution of the test substance with water as is recommended on the proposed label for use of APOLLO® 50 SC in the field. Therefore, an acute inhalation study or data to indicate that the formulation is not in a respirable form under conditions of normal application is needed.

The highest mutation frequency observed in mouse lymphoma cell cultures treated with clofentezine was within a normal range of 4.4 to 15.3 per 10^5 cells. However, no specific information regarding the range such as an average mutation frequency, the number of control groups used to obtain the range, or individual control group values were included. These specific data are needed before the report can be considered complete.

The major route of excretion of clofentezine residues in treated rats is the feces, but metabolites are identified only in the urine. Based on the importance of bile and feces as a route of excretion and the results of long-term feeding studies in rats and mice, identification of fecal metabolites is needed to support a complete assessment of clofentezine toxicity.

2. Reference Dose (RfD)

The lowest NOEL was established in the one-year dog study at 1.25 mg/kg /day, and a Safety Factor of 100 was applied to obtain an RfD of 0.013 mg/kg/day.

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3. Toxicology Branch Peer Review

On September 16, 1987, the Toxicology Branch Peer Review Committee considered the data described in Section I. B. above in a weight-of-the-evidence analysis and classified the oncogenic potential of clofentezine into Category C (possible human oncogen) based on the following:

a. Clofentezine was associated with an increase in tumors (benign and malignant combined) in only one of two species tested, and the tumors occurred in one sex of a single strain of the rat.

b. The observed response exceeded the historical control range, and was observed at the highest dose tested which was well below the Maximum Tolerated Dose (MTD) predicted by subchronic feeding studies.

c. Clofentezine was not mutagenic.

d. The chemical is not structurally related to known carcinogens.

In addition, the available information on specific thyroid effects considered by the Committee (see Section I. B. 10.) did not indicate that the production of thyroid tumors was related to inhibition of thyroid function by clofentezine treatment in the rat.

A quantitative risk assessment was conducted based on the adjusted incidences of combined benign and malignant thyroid tumors in male rats in the 27-month feeding study. The unit risk in human equivalents (multistage model Q_1^*) based on those data was 0.053 $(mg/kg/day)^{-1}$.

4. Scientific Advisory Panel Review

On March 2, 1988, the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) considered the Agency's weight-of-the-evidence considerations and classification of the oncogenic potential of clofentezine in a public meeting. Additional data provided by the Registrant and discussed in Section III. below was also considered by the Panel.

The Panel commented on the biological significance of follicular cell tumors in male rats given clofentezine as follows:

> Thyroid tumors in rats may or may not be biologically significant for humans. If data clearly demonstrate increases in TSH (thyroid stimulating hormone) and the subsequent responses in the thyroid (hyperplasia and neoplasia), this endpoint does not provide evidence of direct, compound-induced carcinogenicity. However, if such data do not exist for other chemicals, thyroid tumors should be considered as evidence for carcinogenic potential.

The Panel agreed with the Peer Review determinations that there were no compound-related effects on tumor incidence in the mouse oncogenicity study and that clofentezine is not mutagenic. However, they did not

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believe that failure to achieve a Maximum Tolerated Dose (MTD) compromises the rat study or that the thyroid tumors in male rats provide evidence of human risk for carcinogenicity. Based on these considerations, the Panel concluded:

> ...(clofentezine) belongs in category D. This interpretation is based primarily on the data demonstrating increased TSH, hyperplasia, and decreased half-life of T_4 and T_3. It is well known that this sequence leads to thyroid tumors in rats. Exposure to agents that cause this sequence in rats has not resulted in increased TSH, hyperplasia, and thyroid tumors in humans. Therefore, there is inadequate data for suggesting human carcinogenicity.

The Panel then concluded that the data were not adequate for a quantitative risk assessment.

II. New Information

A. New Data

Two studies in which male rats were given diets containing 0 or 30,000 ppm clofentezine for two to five weeks followed by an intravenous dose of ^{125}I-thyroxine (Dawson, 1988; and Needham, 1988) showed the following:

1. Bile flow rate in clofentezine treated rats was nearly twice that in control rats.

2. A greater proportion of administered ^{125}I in the bile of pretreated rats was associated with thyroxine metabolites than was observed in untreated control rats.

3. Clofentezine pretreatment altered the route of excretion for thyroxine and decreased the blood levels of exogenous thyroxine below those observed in control rats.

   a. Fecal excretion over a 72 hour period rose from approximately 26% of the administered radioactivity in control rats to 40% in pretreated rats.

   b. Urinary excretion fell from 27% of the administered thyroxine dose to 15%.

   c. The mean concentration of radioactivity in the blood of test rats was reported to be 0.058 and 0.029 ug/kg in the control and pretreated groups, respectively.

Histological re-evaluations of the thyroid glands from male rats in several feeding studies (Ginnochio and Major, 1988a, b, and c; Mallyon and Major, 1988) indicated dose-related increases in the degree of colloid depletion and follicular cell hypertrophy. These observations were noted at dose levels as high as 27,000 ppm, and they were reversible at those dose levels in treated rats after 4 weeks on a control diet (Mallyon and Major, 1988).

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B. Registrant Comments

1. Proposed mechanism for thyroid tumor development

Based on special studies described above, thyroid effects were characterized as an indirect result of enhanced metabolism and clearance of peripheral thyroid hormones in rats (Cochburn, 1988). A direct mechanism for the effects was dismissed for the following reasons:

> ...clofentezine is (1) negative in a full battery of mutagenicity assays, (2) negative in a mouse oncogenicity study, (3) causes no decrease in latency period for thyroid tumor development nor metastases in the rat, (4) does not accumulate preferentially in the thyroid of treated rats, and (5) only increases tumor production at very high dose levels (400 ppm) in rats at which biochemical and endocrine disturbances take place; below this threshold no untoward effects are seen.

The Registrant also noted that several studies suggested the lowest-effect-level for clofentezine's direct effects was 400 ppm (20 mg/kg/day) in rats. That dose and higher levels were associated with elavated mixed-function oxidase enzyme activities which, in three special studies (Needham, Challis, and Campbell, 1981, 1982, and 1983), were shown to be reversible after withdrawal of clofentezine from test diets for two to six weeks.

The Registrant concluded that these direct effects caused an increase in the metabolism and excretion of thyroxine. This increased turnover was associated with a reduction in the level of circulating thyroxine. The pituitary, through the hypothalamus, responds to reduced thyroxine blood levels by secreting thyroid stimulating hormone (TSH). Elevated TSH levels then stimulated follicular cells of the thyroid gland to replenish depleted reserves of the hormone in clofentezine treated rats.

The Registrant noted:

> The thyroid changes resulting from TSH stimulation follow a time-related course of colloid depletion, follicular cell hypertrophy, hyperplasia, adenoma, and carcinoma,...For example, the novel H_2 antagonist SK&F 94379 which increases TSH secretion in rats due to increased T4 clearance has been found to cause characteristic morphological changes culminating in both benign and malignant thyroid tumors after long term administration.

2. Similar effects of other chemicals

Phenobarbitone was characterized as the most widely studied of those chemicals affecting the thyroid gland in a manner similar to that proposed for clofentezine, and its effects in rats were described as follows:

> After large doses (100 mg/kg for several days) have been given to rats to induce increased liver weight, the hepatic uptake of T3 and T4 is markedly increased, and biliary excretion of

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> the hormones rises, owing to increased bile flow and to increased bile:plasma ratios. The deiodinative metabolism of T4, but not of T3, is also enhanced...A marked increase in fecal excretion of T4 results, the net effect of which is to alter tissue hormone levels and to activate TSH secretion.

Specific comments on observations made in the chronic feeding study with rats were as follows:

> ...(after 27 months) Colloid depletion and follicular cell hypertrophy were minimal and this contrasted with the marked effects seen at 400 ppm in interim kill rats from the same study, after 12 months treatment.
>
> The probable reason for this reduction in effect is the known decrease in the percentage induction of microsomal enzymes throughout the adult life-span of the rat. This would in turn progressively reduce the excessive hepatic disposal of thyroid hormones, reduce pituitary feedback and hence reduce the TSH drive on the thyroid gland, with time. Phenobarbitone in particular shows this marked reduction of induced hepatic microsomal enzymes, especially in the second year of life...in the rat carcinogenicity study with clofentezine, centrilobular hepatocyte enlargement was seen in 18/20 interim kill males at 400 ppm but only in 13/50 decedents and terminal kill animals at this dose level. The presence of marginally high serum T4 levels was indicative of a compensatory effect on thyroid function...

3. Species differences and risk extrapolation

Thyroid physiology and biochemistry in the rat and man were cited as further evidence that the thyroid tumors observed in the clofentezine chronic feeding study are not appropriate for risk extrapolation to humans (Cochburn, 1988). The principal differences between the rat and humans were stated as follows:

1. The relatively low binding capacity and afinity of T4 for plasma proteins in the rat.

2. An androgen-mediated sex difference in the rat's regulation of thyroid control which makes the occurrence of thyroid tumors more likely in male than female rats.

According to the Registrant's discussion, the low binding capacity of T4 for plasma proteins in rats is associated with a more rapid turnover of the hormone (a plasma half-life of 12 to 24 hours) than in humans (5 to 9 day plasma half-life). The discussion summarized the importance of the species differences as follows:

> ...hepatic enzyme induction leads to increases in liver T3 and T4 uptake, conjugation and metabolism. Biliary excretion of the thyroid hormones therefore rises owing to increased

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hormone levels in the bile and/or increased bile flow. This compromises the efficiency of enterohepatic recirculation and recovery from the gut of excreted thyroid hormones which is resorbed and rapidly re-excreted in the bile. This process is believed to be less important in the maintenance of the thyroid economy in man than is the case for the rat.

III. Discussion

A. Thyroid Function Studies

Since two experiments demonstrated that clofentezine did not selectively accumulate in the thyroid gland and since iodine uptake was increased in the gland, an indirect effect was investigated. The additional studies showed that short-term administration of high doses of clofentezine (3000 to 30,000 ppm in the diet) caused increased liver enzyme activity and increased bile flow in male rats. Results of two subchronic experiments at 30,000 ppm suggested that these effects changed the metabolism and excretion pattern for thyroid hormone, but the studies did not indicate that these changes would be biologically significant when a lower dose level (400 ppm) is administered chronically.

Enhanced clearance of thyroid hormones could reduce blood levels of thyroxine sufficiently to cause the pituitary gland to secrete thyroid stimulating hormone (TSH) to increase thyroid gland activity. In male rats given diets containing 30,000 ppm clofentezine for 6 weeks, elevated TSH levels were observed, but thyroxine levels were also increased. In addition, the thyroxine levels were elevated in male rats at the end of the chronic study without an increase in their TSH levels. No thyroid blood chemistry tests were conducted during earlier portions of the chronic feeding study, and the 400 ppm dose level in a 6 week feeding study did not significantly increase thyroxine or TSH levels. These circumstances suggest that a longer study over a broader dose range would more clearly characterize the biological significance of clofentezine's effects on the thyroid in male rats.

Microscopic observation of thyroids from rats in chronic and subchronic studies indicated increased activity in the glands of treated male rats. There was a dose-related increase in the severity of colloid depletion along with dose-related increases in the incidence and severity of follicular cell hypertrophy, and an increased incidence of hyperplasia. In short-term studies at high doses (3,000 to 30,000 ppm), follicular cell hypertrophy was reversed in the three weeks following a 9-week treatment period. Observation of hyperplasia was limited to the chronic feeding study, and its incidence was not dose-related. Thyroid weights were increased along with these microscopic changes but not in a statistically significant manner. These marginal changes further emphasize the need for a chronic study using a broader dose range to more clearly support the suggested indirect mechanism for clofentezine's oncogenic potential.

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B. Similarities to other chemicals

The apparent reversal of non-neoplastic changes in older rats in the chronic feeding study suggests that clofentezine is generally similar to phenobarbitone with respect to its non-neoplastic effects on the thyroid gland of male rats (see page 11 above). In addition, the histamine antagonist example (also described on page 11) indicates that other substances enhancing the rate of thyroxine clearance from rats can induce thyroid tumors in that species at toxic dose levels.

C. Peer Review Reconsideration

On May 18, 1988, the Toxicology Branch Peer Review Committee met to assess the SAP review and the available data on thyroid effects (see DER in Appendix III. below for summary of data presented to the Committee). The thyroid data were evaluated according to six indicators of pituitary-thyroid hormone imbalance which are described in a draft document entitled Thyroid Follicular Cell Carcinogenesis: Mechanistic and Science Policy Considerations prepared by the Technical Panel of the Agency's Risk Assessment Forum (dated December 15, 1987). The Peer Review Committee concluded:

1. Goitrogenic activity of clofentezine in vivo included follicular cell hypertrophy and possibly hyperplasia.

2. Clinical chemistry changes did not consistently indicate a pituitary-thyroid hormone imbalance since both TSH and thyroid hormone were increased by a high dose level of clofentezine (30,000 ppm in the diet of rats for 6 weeks).

3. Increased bile flow and a shift toward fecal excretion of thyroxine suggested that clofentezine may enhance clearance of thyroid hormone.

4. Because of the uncertainty regarding clofentezine's induction of follicular cell hyperplasia (point 1. above), a complete progression of lesions showing cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia (benign and possibly malignant tumors) was not demonstrated over the limited dose range in the chronic feeding study (10 to 400 ppm).

5. Follicular cell hypertrophy and colloid depletion were reversed following withdrawal of clofentezine from treated rats.

6. Clofentezine is not related to a class of compounds with chemical structures that show a correlation with thyroid tumor induction.

The Committee concluded that an additional study of greater than 13 weeks' duration with a broader dose range (>400 ppm) with interim thyroid biochemistry and necropsy observations would be needed to more completely define the mechanism of clofentezine's thyroid tumor induction in terms of the six indicators mentioned above. The Committee further concluded that the evidence was not sufficient to change the classification of clofentezine from Categroy C. A quantitative risk assessment based on the thyroid tumor incidence is considered inappropriate by the Committee for the following reasons:

1. The increased tumor incidence was marginally increased above the control incidence (p = 0.048) only at the highest dose tested (400 ppm) in the chronic feeding study.

2. The increased incidence was observed only in male rats.

3. The thyroid tumor incidence in the chronic feeding study's highest dose group (20%) was slightly greater than the historical range provided by limited data (7.5 to 15%; from 2 studies). (Data were adjusted for animals dying prior to appearance of the first thyroid tumor.)

4. The additional thyroid function studies suggest the possibility of an indirect mechanism for follicular cell tumor induction that may be associated with clofentezine's liver toxicity.

IV. References

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APPENDIX I

Toxicology Branch "One-Liners" for
Clofentezine (Tox. Chem. No. 593A)

Tox Chem No. 593 A (NC 21314) File Last Updated 9/10/85 Current Date

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology-rat; FBC Limited; Report no. TOX/82/167-34; August 20, 1982	Technical 100 + % Pure	071085	Teratogenic NOEL > 3200 mg/kg (HDT) Fetotoxic NOEL > 3200 mg/kg/day Maternal NOEL = 1280 mg/kg/day Maternal LEL = 3200 mg/kg/day (differential staining and slight enlargement of the centrilobular hepatocytes) Levels tested by gavage in Charles River COBS CD Sprague-Dawley strain - 0, 320, 1280 and 3200 mg/kg/day		Minimum 003879
Teratology-rabbit; FBC Limited; Report no. TOX/83/167-42; January, 1983	Technical	071714	Teratogenic NOEL > 3000 mg/kg/day (HDT) Fetotoxic NOEL = 1000 mg/kg day Fetotoxic LEL = 3000 mg/kg/day (body wt reduction) Maternal NOEL = 1000 mg/kg/day Maternal LEL = 3000 mg/kg/day (body wt reduction, reduced food intake) Levels tested by gavage in New Zealand strain - 0, 250, 1000 and 3000 mg/kg/day		Minimum 003879
2-Week feeding-rat; FBC Limited; report no. METAB/83/23; 6/15/83	Technical	071914	After two weeks, the 40 and 400 ppm diets increased the cytochrome P_{450} and b_5 concentrations in rats. The 10 ppm diet had no effect on the liver enzymes of the rats. The test diets contained 0, 10, 40, or 400 ppm.		003879

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
8-Week feeding-mice; FBC Limited; report no.METAB /82/20; 4/82	Technical 100%	070965	Specific effects of diets containing 0, 400, or 27,000 ppm on mixed function oxicase activity in the mouse liver were evaluated. The highest dose cuased a 3-fold increase in cytochrome P_{430} and b_5 concentrations, a 2-fold increase in aminopyrene activity, and no change in the aniline hydroxylase activity. Liver weights were increased by approximately 33% by the high dose. The 400 ppm level increased the aminopyrene demethylase activity.		003879
8-Week feeding-rat; FBC Limited; report no.. METAB/82/2; 1/82	Technical	071914	After two weeks on untreated diets, the rats previously fed 27,000 ppm diets showed only slightly elevated cytochrome b_5 levels (see report no. METAB/81/31 above).		003879
8-Week feeding-rat; BFC Limited; report no. METAB/81/31; 11/9/81	Technical 100%	070965	The study specifically evaluated the effects of dietary concentrations of 40 or 27,000 ppm on hepatic mixed-function oxidase activity. The NOEL is 40 ppm, and 27,000 ppm elevated cytochromes P_{450} and b5 as well as aniline hydroxylase activity		003879

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
56-Day oral - baboon; FBC Limited; Report no. TOX/83/167-55; May 19, 1983	Technical	071719	One animal of each sex was used, and the dosage was varied during the experiment. Doses of Emesis in the female when the dose was elevated to four daily doses of 400 mg/kg by gavage. Feces was colored pink as the result of treatment in both animals. No other signs of toxicity were reported. In the context of other studies listed herein this study can be considered as supplementary since its results are consistent with those in other species.		Supplementary 003879
Metabolism - baboon; FBC Limited; Report no. METAB/83/24; 6/10/83	47.7 uCi/g spec. activ. purity unspecified	071714	After a single oral dose of 10 mg/kg urinary metabolites were identified as free and glucuronide conjugated 4-OH NC 21314 (75%). Urinary residues accounted for 5% of the administered dose. No fecal residues were identified.		003879
90-Day feeding-rat; FBC Limited; Report no. TOX/81/167-22; 1/82	Technical 99%	070962	NOEL < 3,000 ppm (LDT) (increase in liver weight, gross changes in livers, and centrilobular enlargement, elevated serum cholesterol and triglyceride levels) The effects were found to be reversible after the 4-week "regression" period. Levels tested in Charles River COBS CD Sprague-Dawley - 0, 3000, 9000 and 27,000 ppm		Supplementary 003879

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Tox Chem No. 593A (NC 21314)

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90-Day feeding-rat; FBC Limited; report no. TOX/ 81/167-23/1; study #81019; 12/81	Technical 99%	070963	The 400 and 4000 ppm diets increased the serum cholesterol levels in male and female rats. A NOEL for centrilobular hepatocellular enlargement was not established according to the original report (see Report no. TOX/ 83/167-44 and Huntingdon Res. Centre studies below) Levels tested in Charles River COBS CD Sprague-Dawley - 0, 40, 400 and 4000 ppm FIRST ADDENDUM #TOX/83/167/-44; Jan 26,1983		Minimum (with addenda) 003879
		071384	Histological examinations of livers from treated rats indicates that 40 ppm is the NOEL with respect to centrilobular hepatocellular enlargement (see report no TOX/81/167-23/1 above) SECOND ADDENDUM; Huntingdon Res.		
		071859	Histopathology of liver treated rats indicates that the 40 ppm level is the NOEL with respect to centrilobular hepatocellular enlargement, and that 400 ppm is the LEL		
90-Day feeding-dog; FBC Limited; report no. TOX/ 81/167-21/1; 12/81	Technical	070964	The NOEL for increased liver weight or electrocardiogram changes was not established (3200 ppm in the diet was the lowest dose tested). No other effects were noted.		Supplementary 003879

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Tox Chem No. 593A (APOLLO, NC 21314)

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
3-Generation reproduction - rat; FBC Limited; Report No. TOX/84/167-66; August 30, 1984	Technical	257994	Reproductive NOEL >400 ppm Systemic NOEL = 40 ppm Systemic LEL = 400ppm (centrilobular hepatocyte hypertrophy, increased liver weight) Dietary levels tested = 0, 4, 40, 400 ppm		Minimum 004651
Chronic-rat; FBC Limited; Report No. TOX/83/167-62; December, 1983	Technical	257993	Interim Report After 18 months of feeding: NOEL = 40 ppm LEL = 400 ppm (slightly increased liver weight and liver-to-body weight ratio, hepatocellular hypertrophy) Dietary levels tested = 0, 10, 40, and 400 ppm (Includes 12-month sacrifice and histopath. on 20 rats/sex/group)		Supplementary 004651
Oncogenic-mice; FBC Limited; Report no. TOX/83/167-64: December, 1983	Technical (98.7% ± 1.3%)	257995	Interim Report No effects observed after 12 months of the study. Dietary levels tested = 0, 50, 500, and 5000 ppm		Supplementary 004651

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Tox Chem No. 593A, NC 21314

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORF Grade/ Doc. No.
1-Year feeding-dog; FBC Limited; report no. TOX/83/167-57; 6/23/83	Technical 98.2%	071714 257493	NOEL = 50 ppm LEL = 1000 ppm (hepatocyte enlargement with eosinophilic cytoplasm; increased liver weight, thyroid & adrenals; elevated serum cholesterol, triglycerides, & alkaline phosphates). Levels tested in beagles: 0, 50, 1000 & 20,000 ppm.		Supplementary 003879 Minimum 004423
Metabolism-rat ; FBC Limited; # METAB/81/26; July, 1981	>98% (sp. act. 86.4 uCi/mg)	070965	50-60% of the an oral dose (0.1 mg/kg) is recovered in the feces of rats during the first 17 hours after dosing. Approximately 20% is recovered during that time in the urine.		003879
Metabolism-rat; FBC Limited; Report no. METAB/83/14; May 3, 1982	>98% (sp. act. 86.4 uCi/mg)	070965	60-70% of an intravenous dose (0.1 mg/kg) is excreted in the feces during the first 24 hours after dosing. Approximately 20-25% is recovered in the urine during that time.		003879
Metabolism-rat; FBC Limited; Report no. METAB/81/38; December, 1981	>98% (sp. act. 86.4 uCi/mg)	070965	The rats were fed a diet containing 27000 ppm for 10 weeks prior to the administration of 0.1 mg/kg i. v. The excretion profile is similar to that reported in study no. METAB/83/14 above.		003879
Metabolism-rat; FBC Limited; Report no. METAB/82/1; January, 1982	>98% (sp. act. 86.4 uCi/mg)	070965	60-70% of an oral dose (10 mg/kg) was excreted in the feces during the first 24 hours after treatment. Approximately 20% was excreted in the urine during that time. The liver and the kidney were found to have the highest tissue concentrations 72 hours after dosing.		003879

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Tox Chem No. 595A, NC 21314

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism - rat; FBC Limited; Report no. METAB/83/27; June 16, 1983.	>98% purity 86.4 mCi spec. activ.	071714	The rats were given a 10 mg/kg dose of radiolabelled test substance. 35% of radioactivity recovered in urine was described as a monochloro-hydroxylated derivative of the parent found free or conjugated. Approximately 34% of the recovered activity was identified as free or conjugated 3- or 4-hydroxy NC 21314. 9% of activity associated with unidentified metabolites and 3% was associated with unmetabolized parent		003879
Metabolism - rat; FBC Limited; Report no. METAB/82/22; March, 1982	>98% purity, spec. activ. 86.4 mCi/g	070965	1000 mg/kg dose. Majority excreted in the feces (98% of administered dose; approximately 1 to 2% of dose excreted in urine. Highest tissue concentrations in the liver and plasma. Moderate levels were found in adrenals and fat. These levels were slightly above those found in all other tissues.		003879
Metabolism - rat; FBC Limited; Report no. METAB/81/32; 11/81	>98% purity 86.4 mCi/g spec. activ.	070965	1-25 consecutive daily doses of 20 mg/kg administered by gavage to rats Tissue levels rose to a plateau in kidney, liver, heart (females only), skin, and ovaries after 5 to 15 days These levels were 2 to 4 times those found after a single dose.		003879
Metabolism - rat; FBC Limited; Report no. METAB/82/23; 4/82	>98% purity 86.4 uCi/g spec. activ.	070965	Whole-body autoradiography of rats given a single 10 mg/kg dose of radiolabelled test substance indicated that there is poor gastrointestinal absorption and organs and tissues are cleared of residues within 48 hours after dosing.		003879

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism - rat; FBC Limited; Report no. METAB/81/19; 4/81	>98% purity 10 uCi/g spec. activ.	070965	Residues in fetuses of rats given a single oral dose of 20 mg/kg were one-fifth the level found in the maternal blood 6 hours after dosing. 24 hours after treatment, the liver, kidneys, and fat had 1 ppm concentrations of radioactive residues. No other tissues contained significant residue concentrations. The test substance did not readily cross the placenta, and is rapidly cleared from pregnant rats.		003879
Metabolism - mice; FBC Limited; Report no. METAB/82/11; 1/82	>98% purity 86.4 uCi/g spec. activ.	070965	Approx. 65% of a single 10 mg/kg oral dose is excreted in the feces of treated mice. Almost all of the administered dose is excreted within 48 hours after treatment.		003879
Metabolism - dog; FBC Limited; Report no. METAB/82/6; January, 1982	>98% purity 86.4 uCi/g spec. activ.	070965	94-97% of the 10mg/kg oral dose was excreted by treated dogs within 48 hours after treatment.		003879
Metabolism - dog; FBC Limited; Report no. METAB/81/37; 12/81	>98% purity 86.4 uCi/g spec. activ.	070965	After an i. v. injection of a single dose of 0.1 mg/kg approximately 70% of the administered radioactivity was recovered in the feces and 20% was found in urine. Most of the excretion was noted during the first 48 hours after dosing. Tissue residues were at or below the level of detection (0.01 ppm).		003879

Tox Chem No. 593A, NC 21314

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism -rabbit; FBC Limited; Report no. METAB/82/21; 4/82	>98% purity 86.4 uCi/g spec. activ.	070965	Approximately 60% of a single oral dose of 10 mg/kg to rabbits was recovered in the feces, and 40% was found in the urine. Almost all of the recovered residues were found during the first 48 hours after dosing. At 96 hours after treatment from 0.5 to 1.43 ppm was found in the stomach, bile, and large intestine of treated rabbits.		003879
Mutagenic - Ames test -Salmonella; FBC Limited; Report no. TOX/80/167-3	Technical 98.5%	070961	No mutagenic activity was found in bacteria with or without metabolic activation at doses ranging from 3.3 mg/plate to 10 ug/plate		003879
Mutagenic (gene conversion and mitotic recombination)-yeast; FBC Limited; Report no. TOX/83/167-56; June 6, 1983	Technical	071714	The vehicle (dimethylformamide/ethanol) was found to be cytotoxic and a reduced concentration had to be used. Concentrations >125 ug per ml culture medium were insoluble. No increases in gene conversions or the frequency of mitotic recombinations were observed at 12.5, 25, 100, or 200 ug/ml above that in vehicle controls was found.		003879
Mutagenic - micronucleus - mice; FBC Limited; report no. TOX/82/167-32; 4/82	Technical	071085	There was no compound-related increase in the number of micronuclei in mice given 800, 1600, or 3200 mg/kg doses above that observed in untreated control mice.		003879
Photosensitization - no species; FBC Limited; Report no. TOX/81/167-17; 5/81	Technical	070961	No data were presented. A theoretical discussion of the potential of the test substance to be phototoxic was presented.		003879

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Tox Chem No. 593A, NC 21314

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - dominant lethal -rat; FBC Limited ; report no. TOX/83/167-45; 3/29/83	Technical	071714	No dominant lethal mutations were induced in rats after 10 weeks of feeding diets containing 0, 4, 40, or 400 ppm. Slightly elevated cholesterol levels, absolute and relative liver weights were observed in rats given the 400 ppm diet.		003879
Acute oral LD_{50}-mice; FBC Limited; Report no. TOX/80/167-7; September, 1980	Technical 99.1%	070961	LD_{50} >3200 mg/kg (only dose tested)	III	Supplementary 003879
Acute oral LD_{50}-dog; FBC Limited; Report no. TOX/80/167-10; June, 1980	Technical 99.1%	070961	LD_{50} >2000 mg/kg (HDT) (only two doses tested and one dog per dose)	III	Supplementary 003879
Acute oral LD_{50}-hamster; FBC Limited; Report no. TOX/80/167-7; September, 1980	Technical 99.1%	070961	LD_{50} >3200 mg/kg (only one dose tested)	III	Supplementary 003879
Acute intraperitoneal LD_{50}-rat; FBC Limited; Report no. TOX/80/167-5; September, 1980	Technical 99.1%	070961	LD_{50} >800 mg/kg		003879
Acute dermal LD_{50}-rat; FBC Limited; Report no. TOX/80/167-4: August, 1980	Technical 98.5%	070961	LD_{50} >1330 mg/kg (only one dose was tested and al animals had intact skin). Sufficiently high doses to clearly establish a Toxicity Category classification were not used.		Supplementary 003879
Acute oral LD_{50} - rat; FBC Limited; Report no. TOX/81/167-15; April, 1981	80 WP (80% a. i.)	070961	LD_{50} > 5000 mg/kg (no mortality)	IV	Minimum 003879

Tox Chem No. 593A,(NC 21314)

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation LC_{50} - rat; FBC Limited; Report no. TOX/82/167-24; 1/82	80 WP (80% a. i.)		LC_{50} > 11.35 mg/L/6 hrs (nominal conc) (no mortality)	III	Minimum 003879
Acute dermal LD_{50} - rabbit; FBC Limited; Report no. TOX/81/167-20; 8/81	80 WP (80% a. i.)	070961	LD_{50} > 20,000 mg/kg (abraded skin)	IV	Minimum 003879
Primary dermal irritation - rabbit; FBC Limited; Report no. TOX/81/167-18; 6/81	80 WP (80% a. i.)	070961	Only slight irritation was observed 24 hours after application. Treated skin appeared normal at 72 hours following treatment. (aqueous saline vehicle used)	IV	Minimum 003879
Primary eye irritation - rabbit; FBC Limited; report no. TOX/81/167-19; 6/81	80 WP (80% a. i.)	070961	The formulation caused mild reversible irritation in washed and unwashed eyes of female rabbits. Conjunctival irritation was the most prominant effect.	IV	Minimum 003879
Acute oral LD_{50} - rat; FBC Limited; Report no. TOX/81/167-12; February, 1981	50 WP (50% a. i.)	070961	LD_{50} > 5000 mg/kg (no mortality)	IV	Guideline 003879
Acute oral LD_{50} - rat; FBC Limited; Report no. TOX/82/167-40; January 19,1983	50 SC (42% a. i.)	071914	LD_{50} > 5000 mg/kg (no mortality)	IV	Guideline 003879
Acute dermal LD_{50} - rabbit; FBC Limited; report no. TOX/83/167-48; 3/7/83	50 SC (42% a. i.)	071714	LD_{50} > 2.4 g/kg (no mortality) (abraded skin)	III	Minimum 003879

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Tox Chem No. 593A (Apollo) File Last Updated _____ Current Date 05/30/86

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acceptable Daily Intake- EPA/ OPP/ HED TOx.	TECH		PADI = 0.013 mg/kg/day Safety Factor = 100 Dated: Updated: Study: 1-Year Feeding Dog Study NOEL: 50 ppm Lab.: FBC Limited Study No.: TOX/83/167-57 Study Date: 6/23/83 Doc.No.: 005096 Comments: Carcinogenicity: Not determined, final report on mouse and rat pending.		005096

TOXCHEM NO. 593A FILE LAST UPDATED: 03/08/88

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Teratology Species: rat FBC Limited TOX/82/167-34; 8/20/82	NC 21314 Tech 100% pure	071085	Teratogenic NOEL > 3200 mg/kg (HDT). Fetotoxic NOEL > 3200 mg/kg/day Maternal NOEL = 1280 mg/kg/day Maternal LEL = 3200 mg/kg/day (differential staining and slight enlargement of the centrilobular hepatocytes) Levels tested by gavage in Charles River COBS CD Sprague-Dawley strain - 0, 320, 1280 and 3200 mg/kg/day		Minimum 003879
Teratology Species: rabbit FBC Limited TOX/83/167-42; 1/83	NC 21314 Tech	071714	Teratogenic NOEL > 3000 mg/kg/day (HDT) Fetotoxic NOEL = 1000 mg/kg day Fetotoxic LEL = 3000 mg/kg/day (body wt reduction) Maternal NOEL = 1000 mg/kg/day Maternal LEL = 3000 mg/kg/day (body wt reduction, reduced food intake) Levels tested by gavage in New Zealand strain - 0, 250, 1000 and 3000 mg/kg/day		Minimum 003879
Reproduction-3 generation Species: rat FBC Limited TOX/84/167-66; 8/30/84	Apollo Tech	257994	Reproductive NOEL >400 ppm Systemic NOEL = 40 ppm Systemic LEL = 400ppm (centrilobular hepatocyte hypertrophy, increased liver weight) Dietary levels tested = 0, 4, 40, 400 ppm		Minimum 004651
Feeding-1 year Species: dog FBC Limited TOX/83/167-57; 6/23/83	NC 21314 Tech 98.2%	071714 257493	NOEL = 50 ppm LEL = 1000 ppm (hepatocyte enlargement with eosinophilic cytoplasm; increased liver weight, thyroid & adrenals; elevated serum cholesterol, triglycerides, & alkaline phosphates). Levels tested in beagles: 0, 50, 1000 & 20,000 ppm.		Supplementary 003879 Minimum 004423
Chronic/onco feeding Species: rat Huntingdon res. Centre, Eng. TOX/84/167-70; 12/17/85	Apollo Tech (98.7% a.i.)	257993 262261 262262	Doses tested: 0, 10, 40 & 400 ppm in Charles River BR str. Syst. NOEL = 40 ppm. Syst. LEL = 400 ppm (incr. liver wts. & incr liver-body wt. ratios (males); incr. thyroxine levels; centrilobular hypertrophy & vacuolation of hepatocytes, focal cystic degeneration of hepatocytes & diffuse distribution of fat deposits in liver (males). Oncogenic NOEL = 40 ppm. Oncogenic LEL = 400 ppm. Incr. incid. of follicular cell tumors (benign &/or malignant) in males.		Supplementary 004651 Minimum 006232
Feeding/oncogenic-2 year Species: mice Huntingdon res. Centre, Eng. TOX/85/167-70; 11/85	Apollo Tech (98.7% a.i.)	257995 262263 262264	Doses tested: 0, 50, 500 & 5000 ppm in CD-1 Swiss str. Syst. NOEL = 500 ppm. Syst. LEL = 5000 ppm (reversible decr in body wt. decr body wt. gain (15 to 22%), incr. incid. of eosinophilic areas or foci of hepatocytes in males; in females incr. incid. of basophilic and/or eosinophilic foci or areas of hepatocytes, an incr. mortality during weeks 78 to 105 with amyloidosis identified as contributing factor to deaths. Oncogenic NOEL = 500 ppm. Oncogenic LEL = 5000 ppm increased incidence of hepatocellular adenomas in females.		Supplementary 004651 Minimum 006232

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TOXCHEM NO. 593A FILE LAST UPDATED: 03/08/88

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Feeding-14 day Species: rat FBC Limited METAB/83/23; 6/15/83	NC 21314 Tech	071914	After two weeks, the 40 and 400 ppm diets increased the cytochrome P450 and b5 concentrations in rats. The 10 ppm diet had no effect on the liver enzymes of the rats. The test diets had: 0, 10, 40, or 400 ppm		003879
Feeding-2 month Species: mice FBC Limited METAB/83/20; 4/82	NC 21314 Tech 100%	070965	Specific effects of diets containing: 0, 400, or 27,000 ppm on mixed function oxidase activity in the mouse liver were evaluated. The highest dose caused a 3-fold increase in cytochrome P430 and b5 concentrations, a 2-fold increase in aminopyrene activity, and no change in the aniline hydroxylase activity. Liver weights were increased by approximately 33% by the high dose. The 400 ppm level increased the aminopyrene demethylase activity.		003879
Feeding-2 month Species: rat FBC Limited METAB/82/2; 1/82	NC 21314 Tech	071914	After two weeks on untreated diets, the rats previously fed 27,000 ppm diets showed only slightly elevated cytochrome b5 levels (see report no. METAB/81/31 above).		003879
Feeding-2 month Species: rat FBC Limited METAB/81/31; 11/9/81	NC 21314 Tech 100%	070965	The study specifically evaluated the effects of dietary concentrations of 40 or 27,000 ppm on hepatic mixed function oxidase activity. The NOEL is 40 ppm, and 27,000 ppm elevated cytochromes P450 and b5 as well as aniline hydroxylase activity		003879
Feeding-2 month Species: baboon FBC Limited TOX//83/167-55; 1983	NC 21314 tech	071719	One animal of each sex was used, and the dosage was varied during the experiment. Emesis in the female when the dose was elevated to four daily doses of 400 mg/kg by gavage. Feces was colored pink as the result of treatment in both animals. No other signs of toxicity were reported. In the context of other studies listed herein this study can be considered as supplementary since its results are consistent with those in other species.		Supplementary 003879
Feeding-3 month Species: rat FBC Limited TOX/81/167-22; 1/82	NC 21314 Tech 99%	070962	NOEL < 3,000 ppm (LDT) (increase in liver weight, gross changes in livers, and centrilobular enlargement, elevated serum cholesterol and triglyceride levels) The effects were found to be reversible after the 4-week "regression" period. Levels tested in Charles River COBS CD Sprague-Dawley - 0, 3000, 9000 and 27,000 ppm		Supplementary 003879

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TOXCHEM NO. 593A FILE LAST UPDATED: 03/08/88

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Feeding-3 month Species: rat FBC Limited 81319; 12/81	NC 21314 Tech 99%	070963 071384 071859	The 400 and 4000 ppm diets increased the serum cholesterol levels in male and female rats. A NOEL for centrilobular hepatocellular enlargement was not established according to the original report (see Report no. TOX/83/167-44 and Huntingdon Res. Centre studies below) Levels tested in Charles River COBS CD Sprague-Dawley - 0, 40, 400 and 4000 ppm. FIRST ADDENDUM #TOX/83/167/-44; Jan 26,1983 Histological examinations of livers from treated rats indicates that 40 ppm is the NOEL with respect to centrilobular hepatocellular enlargement (see report no TOX/81/167-23/1 above). SECOND ADDENDUM; Huntingdon Res. Histopathology of liver treated rats indicates that the 40 ppm level is the NOEL with respect to centrilobular hepatocellular enlargement, and that 400 ppm is the LEL		Min (w addenda) 003879
Feeding-3 month Species: dog FBC Limited TOX/81/167-21/1; 12/81	NC 21314 Tech	070964	The NOEL for increased liver weight or electrocardiogram changes was not established (3200 ppm in the diet was the lowest dose tested). No other effects were noted.		Supplementary 003879
Feeding-6 week Species: rat FBC Limited TOX/85/167-77; 1/27/86	Apollo Tech (99.3 +- 1%)	262268	Doses tested: 0, 400, & 30,000 ppm in diet, Charles River str. NOEL = 400 ppm (LDT). (Incr. liver wt., & liver to body weight ratios (males); dehydroepiandrosterone (females); at 30,000 ppm incr. thyroxine free thyroxine index, thyrotrophin, progesterone & dehydroepiandrosterone in both sexes; males also had incr. triiodothyroxine		Supplementary 000632
Metabolism Species: baboon FBC Limited METAB/83/24; 6/10/83	NC 21314 47.7 uCi/g spec. activ purity unspecified	071714	After a single oral dose of 10 mg/kg urinary metabolites were identified as free and glucuronide conjugated 4-OH NC 21314 (75%). Urinary residues accounted for 5% of the administered dose. No fecal residues were identified.		003879
Metabolism Species: rat FBC Limited METAB/81/26; 7/81	NC 21314 > 98% (sp. act. 86.4 uCi/mg)	070965	50-60% of the an oral dose (0.1 mg/kg) is recovered in the feces of rats during the first 17 hours after dosing. Approximately 20% is recovered during that time in the urine.		003879
Metabolism Species: rat FBC Limited METAB/83/14; 5/3/82	NC 21314 > 98% (sp. act. 86.4 uCi/mg)	070965	60-70% of an intravenous dose (0.1 mg/kg) is excreted in the feces during the first 24 hours after dosing. Approximately 20-25% is recovered in the urine during that time.		003879
Metabolism Species: rat FBC Limited METAB/81/38; 12/81	NC 21313 > 98% (sp. act. 86.4 uCi/mg)	070965	The rats were fed a diet containing 27000 ppm for 10 weeks prior to the administration of 0.1 mg/kg i. v. The excretion profile is similar to that reported in study no. METAB/83 /14 above.		003879

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Metabolism Species: rat FBC Limited METAB/82/1; 1/82	NC 21314 > 98% (sp. act. 86.44 uCi/mg)	070965	60-70% of an oral dose (10 mg/kg) was excreted in the feces during the first 24 hours after treatment. Approximately 20% was excreted in the urine during that time. The liver and the kidney were found to have the highest tissue concentrations 72 hours after dosing.		003879
Metabolism Species: rat FBC Limited METAB/83/27; 6/16/83	NC 21314 > 98% (sp. act. 86.4 mCi)	071714	The rats were given a 10 mg/kg dose of radiolabelled test substance. 35% of radioactivity recovered in urine was described as a monochloro-hydroxylated derivative of the parent found free or conjugated. Approximately 34% of the recovered activity was identified as free or conjugated 3- or 4-hydroxy NC 21314.9% of activity associated with unidentified metabolites and 3% was associated with unmetabolized parent		003879
Metabolism Species: rat FBC Limited METAB/82/22; 3/82	NC 21314 > 98% (sp. act. 86.4 mCi/g)	070965	1000 mg/kg dose. Majority excreted in the feces (98% of administered dose; approximately 1 to 2% of dose excreted in urine. Highest tissue concentrations in the liver and plasma. Moderate levels were found in adrenals and fat. These levels were slightly above those found in all other tissues.		003879
Metabolism Species: rat FBC Limited METAB/81/32; 11/81	NC 21314 > 98% (sp. act. 86.4 mCi/g)	070965	1-25 consecutive daily doses of 20 mg/kg administered by gavage to rats Tissue levels rose to a plateau in kidney, liver, heart (females only), skin, and ovaries after 5 to 15 days. These levels are 2 to 4 times those found after a single dose.		003879
Metabolism Species: rat FBC Limited METAB/82/23; 4/82	NC 21314 > 98% (sp. act. 86.4 uCi/g)	070965	Whole-body autoradiography of rats given a single 10 mg/kg dose of radiolabelled test substance indicated that there is poor gastrointestinal absorption and organs and tissues are cleared of residues within 48 hours after dosing.		003879
Metabolism Species: rat FBC Limited METAB/81/19; 4/81	NC 21314 > 98% (sp. act. 10 uCi/g)	070965	Residues in fetuses of rats given a single oral dose of 20 mg/kg were one-fifth the level found in the maternal blood 6 hours after dosing. 24 hours after treatment, the liver, kidneys, and fat had 1 ppm concentrations of radioactive residues. No other tissues contained significant residue concentrations. The test substance did not readily cross the placenta, and is rapidly cleared from pregnant rats.		003879
Metabolism Species: mice FBC Limited METAB/82/11; 1/82	NC 21314 > 98% (sp. act. 86.4 uCi/g)	070965	Approx. 65% of a single 10 mg/kg oral dose is excreted in the feces of treated mice. Almost all of the administered dose is excreted within 48 hours after treatment.		003879
Metabolism Species: dog FBC Limited METAB/826; 1/82	NC 21314 > 98% (sp. act. 86.4 uCi/g)	070965	94-97% of the 10mg/kg oral dose was excreted by treated dogs within 48 hours after treatment.		003879

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Metabolism Species: dog FBC Limited METAB/81/37; 12/81	NC 21314 > 98% (sp. act. 86.4 uCi/g)	070965	After an i. v. injection of a single dose of 0.1 mg/kg approximately 70% of the administered radioactivity was recovered in the feces and 20% was found in urine. Most of the excretion was noted during the first 48 hours after dosing. Tissue residues were at or below the level of detection (0.01 ppm).		003879
Metabolism Species: rabbit FBC Limited METAB/82/21; 4/82	NC 21314 > 98% (sp. act. 86.4 uCi/g)	070965	Approximately 60% of a single oral dose of 10 mg/kg to rabbits was recovered in the feces, and 40% was found in the urine. Almost all of the recovered residues were found during the first 48 hours after dosing. At 96 hours after treatment from 0.5 to 1.43 ppm was found in the stomach, bile, and large intestine of treated rabbits.		003879
Dermal absorption Species: rat Huntingdon res. Centre, Eng. METAB/86/1; 2/3/86	Apollo radiolabelled > 90%, sp. act. 47.4 mCi /g) in a simulated SC formulation	262268	Doses tested: 4.8, 44, or 180 mg/kg applied to skin of male rats. Less than 1% was absorbed through the skin during the 10 hr exposure pd.		Acceptable 006232
Metabolism Species: rat FBC Limited METAB/85/2; 3/15/85	Apollo radiolabelled (> 99%, sp. act. 47.7)	262268	Doses tested: 1000 mg/kg. Peak levels in plasma were 15.6 ppm for males & 14.1 ppm for females. These peak levels were observed 6 to 8 hours after dosing and declined to approx. 3 ppm, 24 hrs after dosing. Plasma half life = 3.6 hrs. pprox. 40% of radioactivity is associated with metabolites of test substance.		Acceptable 006232
Metabolism Species: rat FBC Limited METAB/85/36; 12/20/85	Apollo radiolabelled (>99%, sp. act. 47.7 mCi/g)	262268	No accumulation of radioactivity in the thyroid after 1 or 10 daily oral doses of 20 mg Apollo/kg body wt. in rats. Before dietary dosing, thyroxine half-lives for control and treated groups of male rats were 16.7 and 17.05 hrs, respectively. Those values after a 4 week feeding period (0 or 30,000 ppm) were 17.61 and 16.42 hrs. After 4 weeks on diets containing 0 or 30,000 ppm, iodine uptake by thyroid in male and female rats was increased and blood levels were decreased.		Acceptable 006232
Metabolism Species: mice FBC Limited METAB/85/36; 12/20/85	Apollo radiolabelled (99% sp. act. 47.7 mCi/g)	262268	In mice, blood iodine levels were comparable & thyroid iodine levels were higher in treated mice when compared with untreated mice.		Acceptable 006232
Photosensitization Species: ? FBC Limited TOX/81/167-17; 5/81	NC 21314 Tech.	070961	No data were presented. A theoretical discussion of the potential of the test substance to be phototoxic was presented.		003879

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Mutagenic-Ames Species: salmonella FBC Limited TOX/80/167-3	NC 21314 Tech 98.5%	070961	No mutagenic activity was found in bacteria with or without metabolic activation at doses ranging from 3.3mg/plate to 10 ug/plate		003879
Mutagenic-gene conv/mit recomb Species: yeast FBC Limited TOX/83/167-56; 6/6/83	NC 21314 Tech	071714	The vehicle (dimethylformamide/ ethanol) was found to be cytotoxic and a reduced concentration had to be used. Concentrations >125 ug per ml culture medium were insoluble. No increases in gene conversions or the frequency of mitotic recombinations were observed at 12.5, 25, 100, or 200 ug/ml above that in vehicle controls was found.		003879
Mutagenic-micronucleus assay Species: mice FBC Limited TOX/82/167-32; 4/82	NC 21314 tech	071085	There was no compound-related increase in the number of micronuclei in mice given 800, 1600, or 3200 mg/kg doses above that observed in untreated control mice.		003879
Mutagenic-dominant lethal test Species: rat FBC Limited TOX/83/167-45; 3/29/83	NC 21314 Tech	071714	No dominant lethal mutations were induced in rats after 10 weeks of feeding diets containing 0, 4, 40, or 400 ppm. Slightly elevated cholesterol levels, absolute and relative liver weights were observed in rats given the 400 ppm diet.		003879
Mutagenic- lymphoma mutation Species: mice Life Science Research TOX/82/167-38; 10/14/82	Apollo Tech. (98.8% +- 1.4%)	262268	Doses tested: 15, 30, 70, 100 & 128 ug/ml without microsomal activ.; 2, 10, 30, 80, 100, & 128 ug/ml with activation. No increase in frequency of mutation at the TK locus in L5178Y cells in vitro. Highest dose tested doubled the mutation frequency above concurrent control cultures, but report stated that frequency was within historical range. No data on historical controls were included.		Provis. Accep. 006232

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APPENDIX II

DATA EVALUATION RECORDS
for New Toxicology Data

Dawson, J. January 5, 1988. The Effect of the Dietary Administration of Clofentezine on the Biliary Excretion of a Single Intravenous Dose of L[^{125}I]-Thyroxine Unpublished report no. ENVIR/87/49 prepared by Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-07.

Needham, D. January 5, 1988. The Effect of the Dietary Administration of Clofentezine on the Excretion of a Single Intravenous Dose of L[^{125}I)-Thyroxine. Unpublished report no. ENVIR/87/50 prepared by Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE MRID No. 404679-08

Mallyon, B. A., and I. R. Major January 5, 1988. Technical Clofentezine: 6 Week Dietary Investigation of Thyroid Function in the Rat. (Histopathology review of the thyroid gland) Supplement to Original Report. Unpublished report nos. TOX/85/167-77 and TOX 85014 Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-03

Mallyon, B. A., and L. P. Markham. January 5, 1988. Technical NC 21314: Investigation into Effects on the Thyroid in the Rat. Unpublished report nos. TOX/87/167-98, and TOX 87293 prepared by Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-09

Ginocchio, A. V., and I. R. Major. January 5, 1988. Unpublished report nos. TOX/84/167-70 and TOX 82003 prepared by Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-06.

Ginocchio, A. V., and I. R. Major. January 5, 1988. Technical NC 21314: 90-Day Dietary Toxicity Study in the Rat (Histopathology review of the thyroid gland) Supplement to Original Report. Unpublished report nos. TOX/81/167-22 and TOX 81001 prepared by Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-04.

Ginocchio, A. V., and I. R. Major. January 5, 1988. The 90-Day Dietary Toxicity Study of Technical NC 21314 (CR 20099/5, Pilot Plant) to the Male and Female Rat (Histopathology review of the thyroid gland). Supplement to the Original Report. Unpublished report nos. TOX/81/167-23/1 and TOX 81019 prepared by Schering Agrochemicals Ltd. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-05.

Capen, C. September 1, 1987. Histopathologic Evaluation of Thyroid and Parathyroid Glands from Male and Female Sprague-Dawley Rats Fed Clofentezine and Benazolin-Ethyl. Unpublished report no. TOX 82003 prepared by Ohio State University, Department of Veterinary Pathology. Submitted by Nor-Am Chemical Co., Wilmington, DE. MRID No. 404679-10

Reviewed by: Roger Gardner R.G. 5-6-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 5/9/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Biliary excretion of thyroid hormone

MRID NUMBER: 404679-07

TEST MATERIAL: Technical grade Clofentezine described as a magenta crystaline powder (99.3% purity; lot no. CR 20099/15).

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): ENVIR/87/49

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT: The Effect of the Dietary Administration of Clofentezine on the Biliary Excretion of a Single Intravenous Dose of L[^{125}I]-Thyroxine

AUTHOR(S): Dawson, J.

REPORT ISSUED: January 5, 1988

CONCLUSIONS: Two groups of male Sprague-Dawley strain rats were given diets containing 0 or 30,000 ppm clofentezine for 2 to 3 weeks. At the end of that time, the test animals were given an intra-venous dose of ^{125}I-thyroxine, and bile and blood samples were collected at intervals during the 4 hours that followed administration of the hormone.

There are adequate data presented in the report to indicate that clofentezine increases the bile flow and thyroxine metabolism in male rats.

The report indicated that, immediately after dosing, blood levels of ^{125}I were higher in clofentezine-pretreated rats suggesting that those animals could have received a higher dose of ^{125}I-thyroxine. Since no individual animal data were presented in the report, the significance of the greater amount of hormone in pretreated animals with respect to conclusions about excretion rates, blood clearance rates, and cumulative excretion of radiolabel could not be characterized.

Core classification: Supplementary. The study was conducted to investigate the effects of clofentezine on the thyroid of male rats rather than to establish no-effect levels, and the data as reported were not sufficient to unequivocally support the conclusions discussed below (Section III.).

I. PROTOCOL

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A. MATERIALS

1. Test species: Male Charles River Sprague-Dawley strain rats were used. They weighed from 198 to 228 g at the start of the study.

2. Radiolabeled substances: L-[I^{125}]-thyroxine with a specific activity of 200 uCi/ml (in an ethanol/water solution) was used. Radioactive half-life was stated to be 60 days.

3. Diet preparation: Basal diet consisted of Rat and Mouse Diet No. 1 supplied by SDS, Ltd.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

No.	Test groups Designation	Dose (ppm)	Animals per group
1	Control	0	6
2	High (HDT)	30,000	6

These diets were fed to the test animals for 2-3 weeks.

2. Animal observations: After 2 to 3 weeks on test diets, each animal was weighed, anesthetized, and the bile duct was cannulated. Anesthesia was maintained throughout the sampling period (approximately 4 hours), and bile samples were collected (including a blank sample just before administration of thyroxine).

Approximately 100 ng of L[I^{125}]-thyroxine (5 ug/kg) was injected intravenously in the tail vein. Blood samples were taken as soon as possible after dosing and at 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes after the thyroxine was administered.

The animals were sacrificed at the end of the 4-hour sampling period.

3. Analysis of samples: Bile samples were collected, weighed, and counted directly. Counts per ml of bile and cumulative excretion of ^{125}I were calculated.

Blood samples were collected and counted directly. The amount of radioactivity was determined and expressed as mg equivalents.

The report stated that bile samples collected at 45-60, 105-120, 165-180, and 225-240 minutes after treatment with thyroxine were prepared for thin layer chromatography. The pH of each 150 ul aliquot of these samples was adjusted with 6 N HCl and a solution of beta-glucuronidase (5000 units/ml) was added. These samples were incubated at least 16 hours at 37° C.

3. Analysis of samples (continued)

Twenty ul aliquots of the hydrolyzed and unhydrolyzed bile were spotted on silica gel thin layer chromatography (TLC) plates along with standard solutions of thyroxine (T4) and tri-iodothyroxine (T3). The plates were then developed with a butanol:ethanol:2 N ammonium hydroxide (6:3:1) solvent system. The radioactivity on the developed plates was then quantified with a TLC linear analyzer to determine the amount of T4 present.

II. REPORTED RESULTS

According to the report, the bile flow rate in treated rats was approximately double that observed in untreated rats (see Figure 1 in the Addendum below). Four hours after administeration of the radiolabeled thyroxine, the clofentezine treated rats excreted 10.2% of the ^{125}I in comparison to 6.4% for the control group rats (see Figure 2 in the Addendum below). The biliary concentration of ^{125}I-thyroxine in treated animals was higher than that in control animals only during the first 30 minutes following administration. Thereafter, the concentration of ^{125}I in the bile from treated rats was lower than that in control rats (see Figure 4 in the Addendum below).

The report noted that the blood level of radiolabeled thyroxine was higher in treated rats than it was in control animals just after dosing, and the rate of blood clearance during the first 15 minutes following administration of thyroxine was higher in treated rats (2.20 ml/hr compared with 1.45 ml/hr in control animals) (see Figure 3 in the Addendum below). The report further stated that the clearance rates beyond two hours after thyroxine administration were similar in the control and treated groups.

According to the report, the profile of thyroxine metabolites in the bile was changed by clofentezine pretreatment. The report indicated that the proportion of excreted radioactivity represented by thyroxine was less in clofentezine treated animals than that in control rats (see Addendum below for tabulated group mean values). The proportion of excreted radioactivity represented by the glucuronide conjugate of thyroxine was higher in treated rats than in the control group animals during the first two hours following administration of radiolabeled thyroxine. After that time the proportion of excreted activity represented by the conjugate in treated rats was less than that for control group animals.

The total amount of ^{125}I excreted in the bile was higher at all time points for treated rats than control animals during the 4-hour observation period, but the proportion of radioactivity excreted as thyroxine was lower for clofentezine pretreated rats than the control animals.

II. DISCUSSION

Investigators' conclusions: The report concluded:

> Dietary dosing of clofentezine at 30000 ppm to male rats for 2-3 weeks resulted in the doubling of bile flow rate and an increase in the excretion rate of an intra-venous dose of (^{125}I)-thyroxine (1.6 fold). The initial blood clearance rate was also increased by more than 50% when compared to control animals.
>
> The (^{125}I) excreted into bile of control rats contained a higher level of thyroxine and thyroxine glucuronide than did the bile of treated rats. This indicated a higher level of alternative metabolic routes being prevalent in the livers of treated rats, which excreted a higher overall level of (^{125}I) into bile than control rats.
>
> The results obtained all indicate that the effect which clofentezine has on the thyroid gland is due to an increased turnover of the thyroid hormones, resulting in an increased biliary excretion of metabolites of thyroxine. The effects of clofentezine on thyroid hormone turnover are most likely caused by the induction of various hepatic enzymes which are responsible for the catabolism of thyroxine.

Reviewer's Discussion: The observations of greater blood clearance rate for radiolabeled thyroxine in pretreated rats and increased cummulative biliary excretion of ^{125}I thyroxine and metabolites in those rats suggest that clofentezine pretreatment increases the turnover rate of thyroxine in male rats. However, the report stated that blood levels observed in samples collected immediately after administration of ^{125}I-thyroxine were higher in pretreated rats than they were for controls. Therefore, the observations of increased clearance rate and increased bile concentrations in pretreated rats could also be the result of administration of a higher dose of ^{125}I-thyroxine which the pretreated rats excrete in order to maintain the thyroxine level attained during the pretreatment period.

Results supporting the second possibility include the observation that bile concentrations were higher in pretreated rats only during the first 30 minutes following administration of ^{125}I-thyroxine. For the remaining 3.5 hours of the sampling period bile levels of ^{125}I were lower in pretreated rats than in controls.

The report did not include ^{125}I measurements in the livers of pretreated and control group rats to support the conclusion that liver enzyme induction increased thyroxine uptake and metabolism in that organ, but the decreased proportion of excreted ^{125}I in bile samples represented by thyroxine suggests that thyroxine is metabolized at a greater rate in clofentezine pretreated rats.

Reviewer's Discussion (continued)

Individual data were not presented in the report to determine the effect of the higher blood levels of ^{125}I-thyroxine noted immediately after its administration on the blood clearance rates, the initially elevated bile ^{125}I concentrations, or the apparently increased thyroxine metabolism observed.

There are adequate data presented in the report to indicate that clofentezine increases the bile flow and thyroxine metabolism in male rats.

ADDENDUM

Results as Reported on Blood Levels, Bile Levels, and Relative Bile Concentrations of Thyroxine and Its Metabolites in Male Rats

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CLOFENTEZINE TOXICOLOGY REVIEW

Page _____ is not included in this copy.

Pages 44 through 50 are not included in this copy.

The material not included contains the following type of information:

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___ Description of product quality control procedures

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X FIFRA registration data

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006783

Reviewed by: Roger Gardner a.b. 7-13-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 7/13/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Excretion of thyroid hormone

MRID NUMBER: 404679-08

TEST MATERIAL: Technical grade Clofentezine described as a magenta crystaline powder (99.3% purity; lot no. CR 20099/15).

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): ENVIR/87/50

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT: The Effect of the Dietary Administration of Clofentezine on the Excretion of a Single Intravenous Dose of L[^{125}I]-Thyroxine

AUTHOR(S): Needham, D.

REPORT ISSUED: January 5, 1988

CONCLUSIONS: Groups of 5 male rats were given diets containing 0 or 30,000 ppm clofentezine for 5 weeks. At the end of the feeding period, the rats were given an intravenous dose of ^{125}I-thyroxine, and placed into metabolism cages. Urine and feces were collected at intervals during the 72-hour period following administration of the thyroxine, and at the end of the 72-hour observation period the rats were sacrificed. Blood samples were collected, and the gastrointestinal tract was removed for analysis.

The results of the study suggested that clofentezine pretreatment might alter the route of excretion for thyroxine. Fecal excretion over a 72 hour period rose from approximately 26% of the administered radioactivity in control rats to 40% in pretreated rats, and urinary excretion fell from 27 to 15% of the administered thyroxine dose. The mean concentration of radioactivity in the blood of test rats was reported to be 0.058 and 0.029 ug/kg in the control and pretreated groups, respectively. These results suggested that clofentezine pretreatment increased the excretion of thyroxine in male rats.

The toxicological significance of these results could not be determined from the data as reported (see "Reviewer's discussion" in Section III. below).

Core classification: Supplementary. The study was designed to evaluate a specific effect of clofentezine on the thyroid gland of male rats.

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I. PROTOCOL

006783

A. MATERIALS

1. Test species: Male Charles River Sprague-Dawley strain rats were used. They weighed from 198 to 228 g at the start of the study.

2. Radiolabelled substances: L-[I^{125}]-thyroxine with a specific activity of 200 uCi/ml (in an ethanol/water solution) was used. Radioactive half-life was stated to be 60 days.

3. Diet preparation: Basal diet consisted of Rat and Mouse Diet No. 1 supplied by SDS, Ltd.

4. Tissue solubilizer: The SHT solubilizer consisted of 80 g sodium hydroxide in 100 ml Triton X-405, 300 ml methanol, and 600 ml water.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

No.	Test groups Designation	Dose (ppm)	Animals per group
1	Control	0	6
2	High (HDT)	30,000	6

These diets were fed to the test animals for approximately 5 weeks.

2. Animal observations: After 5 weeks on test diets, each animal was weighed, and approximately 100 ng of L[I^{125}]-thyroxine (5 ug/kg) was injected intravenously in the tail vein. The animals were placed in metabolism cages, and their urine and feces were collected at 3, 6, 9, 12, 24, 28, 32, 48, 54, and 72 hours after the thyroxine was administered.

At the end of the observation period the animals were anesthetized and blood was collected from the aorta. They were then sacrificed.

3. Analysis of samples: Aliquots of 0.1 to 1.0 ml urine or blood were placed into counting tubes. Feces were homogenized in water and approximately 1 g aliquots were weighed into counting tubes. At sacrifice of test rats the gastrointestinal tract was removed and solubilized in SHT tissue solubilizer. The report noted that 1 g aliquots of the solubilized G. I. tract tissue were put into counting tubes.

II. REPORTED RESULTS

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Table 1 summarizes the mean percentage of administered radioactivity recovered in urine and feces for the two test groups.

Table 1

Summary of ^{125}I-thyroxine Excretion (expressed as per cent of administered radioactivity) in Male Rats*

Observation time (hours)	Urine		Feces	
	Control	Pretreated	Control	Pretreated
3	0.35	0.71	-	0.00
6	1.77	1.19	-	0.00
9	0.39	0.22	-	0.00
12	2.43	2.03	-	0.78
24	7.68	3.97	3.69	7.33
28	0.43	0.21	-	3.57
32	3.38	0.98	1.60	4.12
48	4.96	3.66	10.42	13.06
54	2.07	0.73	1.06	2.54
72	3.74	1.61	11.48	14.99
Total	27.23	15.31	26.45	40.40

*No statistical analysis was performed on these data.

The amount of radioactivity recovered from the gastrointestinal tract (expressed as a percentage of the administered ^{125}I) was 3.03% for the control group and 2.57% for the clofentezine pretreated group. Total recoveries were 56.71 and 58.27% for the control and pretreated groups, respectively.

The mean concentration of radioactivity in the blood of test rats was reported to be 0.058 and 0.029 ug/kg.

Curves for cummulative excretion in urine, feces, and total excretion are included in the Addendum below.

III. DISCUSSION

Investigators' conclusions: The investigators stated that clofentezine pretreatment at a dietary level of 30,000 ppm for 5 weeks altered the route of excretion for thyroxine. Fecal excretion over a 72 hour period rose from approximately 26% of the administered radioactivity in control rats to 40% in pretreated rats, and urinary excretion fell from 27 to 15% of the administered thyroxine dose.

Reviewer's discussion: Although the results as reported suggest that total excretion of exogenous thyroxine is increased from approximately 56 to 58% of the administered radioactivity, the biological significance of these results is difficult to judge because the study is limited in scope.

Reviewer's discussion (continued)

Its design is limited to only 5 animals per group, and approximately 40% of the administered radioactivity is unaccounted for.

The variability of measurements reported in the individual animal data is greater in the pretreated animals than in controls (total recovery ranging from 49.98 to 67.45% of the administered dose for pretreated animals; total recovery in control group animals ranging from 52.59 to 61.84%). The medians for the two groups are 58.22 and 57.22% for the control and pretreated groups, respectively. With this kind of variation and the limited numbers of animals used in the experiment, statistical analysis may be misleading, especially without some measure of the recovery efficiency for the methods used in the study.

In addition, the tissue distribution of thyroxine is not limited to the gastrointestinal tract, and other tissues should have been analyzed to account for a larger proportion of the administered radioactivity. The liver is of particular interest since it is the organ the investigators have implicated in the metabolism and excretion of the administered ^{125}I-thyroxine.

Based on these considerations, this study can not be used to determine the toxicological significance of the change in the route of thyroxine excretion caused by clofentezine pretreatment at 30,000 ppm in the diet of male rats for five weeks.

ADDENDUM

Cummulative Excretion Data as Presented in a Report on
Clofentezine Pretreated Rats

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Reviewed by: Roger Gardner R. G. 5-6-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 5/9/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic feeding study supplement

MRID NUMBER: 404679-03

TEST MATERIAL: Technical grade Clofentezine (see Addendum)

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): TOX/85/167-77; TOX 85014

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT: Technical Clofentezine: 6 Week Dietary Investigation of Thyroid Function in the Rat. (Histopathology review of the thyroid gland) Supplement to Original Report.

AUTHOR(S): Mallyon, B. A., and I. R. Major

REPORT ISSUED: January 5, 1988

DISCUSSION AND CONCLUSIONS: There were adequate data presented in the re-evaluation of thyroid tissue sections to support the conclusions of the supplemental report. The investigators concluded that there was a clear effect on colloid depletion and follicular cell histology in the thyroid of high dose group male and female rats. The lowest effect level (LEL) was 30,000 ppm and the no-observed-effect level (NOEL) for those effects was 400 ppm.

Core Classification: Supplementary. The report was an ammendment to a previously submitted study.

I. BACKGROUND

This supplemental report describes a re-evaluation of thyroid tissue sections from a subchronic study designed to further investigate effects observed at the end of a long-term feeding study in rats given diets containing up to 400 ppm clofentezine for 27 months [Ginnocchio, A. V.; and Mallyon, B. A. December 17, 1985. The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofentezine in the Diet to the Rat (Final Report). Unpublished Report No. TOX/84/167-70 prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.]. No attempt was made to establish a NOEL. The Data Evaluation Record on the 6-week study is included in Addendum I below.

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II. MATERIALS AND METHODS

The thyroid sections originally stained with hematoxylin and eosin were recoded and examined by another pathologist. The tissue sections were taken from 30 animals given diets containing 0, 400, or 30,000 ppm clofentezine for six weeks.

The report noted that the right and left lobes were examined separately and scored according to the following:

1. Colloid depletion - a reduction in the overall luminal size and staining intensity (degree of eosinophilia) of the colloid.

2. Follicular cell enlargement - cells lining the lumen of the follicle were either squamous in the resting follicles, low cuboidal, cuboidal or columnar in highly active follicles, and lobes with a greater proportion of cuboidal and columnar cells were given a higher score for this condition.

Numerical scores were given for these conditions as follows: 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe.

Results were analyzed by the Kruskal-Wallis Rank test.

III. REPORTED RESULTS

Tabulated results as reported are included in Addendum II below.

The investigators noted that all five control group males scored moderate for colloidal depletion. Two of the five males in the high dose group showed severe depletion, and the remaining three in the group had at least slight depletion. In the low dose group, the authors noted that one of the five males exhibited severe depletion and two had less than slight colloid depletion. The remaining two low-dose group males had moderate colloid depletion. The investigators characterized the results in the control group males as unusual, but they concluded that there was a clear effect on colloid at the 30,000 ppm dose and an equivocal effect in the low dose males.

The investigators also described the results in female rats as unusual but not to the degree observed in males. They also concluded that the high dose had a clear effect on colloid depletion in female rats, and no apparent effect was observed in the low dose group females.

Results with respect to effects on follicular cells were similar to those with colloid depletion according to the investigators.

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ADDENDUM I

Data Evaluation Record for

Saunders, P. C., and B. A. Mallyon. January 27, 1986. Technical Clofentezine: 6 Week Dietary Investigation of Thyroid Function in the Rat. Unpublished report no. TOX/85/167-77 prepared by FBC Limited. Submitted by Nor-Am Chemical Co.; EPA Acc. No. 262268.

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DATA EVALUATION RECORD

CHEMICAL NAME: NC 21314, Clofentezine, APOLLO® 3,6-bis[2-chlorophenyl]1,2,4,5-tetrazine

STUDY/ACTION TYPE: Subchronic feeding study (6 weeks) - rat

STUDY IDENTIFICATION: Saunders, P. C., and B. A. Mallyon. January 27, 1986. Technical Clofentezine: 6 Week Dietary Investigation of Thyroid Function in the Rat. Unpublished report no. TOX/85/167-77 prepared by FBC Limited. Submitted by Nor-Am Chemical Co.; EPA Acc. No. 262268.

REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 8-7-87

APPROVED BY:

Name: Judith Hauswirth, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Judith W. Hauswirth
Date: 8/21/87

DISCUSSION AND CONCLUSIONS: Male and female rats were fed diets containing 0, 400, or 30,000 ppm clofentezine for 6 weeks in an experiment to investigate the pesticide's effect on thyroid function.

The test substance increased absolute liver weights and liver-to-body weight ratios for both sexes at the 30,000 ppm level and for males given the 400 ppm diet.

Increases in thyroxine, free thyroxine index, thyrotrophin, progesterone, and dehydroepiandrosterone were seen in both sexes at 30,000 ppm. Males given the highest dose also showed an increased level of tri-iodothyroxine. At the 400 ppm dose level, males also had increased thyroxine levels, and females had increased dehydroepiandrosterone levels.

Based on the liver weight and hormone observations, a no-observed-effect level (NOEL) was not established in this experiment.

Background: This study was conducted to investigate effects observed at the end of a long-term feeding study in rats given the highest does tested (400 ppm) [Ginnocchio, A. V.; and Mallyon, B. A. December 17, 1985. The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofentezine in the Diet to the Rat (Final Report). Unpublished Report No. TOX/ 84/167-70 prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.]. No attempt was made to establish a NOEL.

Core Classification: Supplementary based on the stated purpose of the study which was to investigate the specific nature of clofentezine's effect on thyroid function.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test species: Male and female Charles River CR1: COBS CD (SD) BR Sprague-Dawley rats were used. They were 28 days of age on arrival at the laboratory conducting the experiment, and body weights ranged from 66 to 85 g for males and 52 to 74 g for females.

2. Test substance: Technical grade clofentezine (Batch no. 20099/14, 99.3 ± 1.0% purity) were used.

3. Diet preparation: Basal diet consisted of Modified SQC Expanded Rat and Mouse Maintenance Diet No. 1 supplied by Special Diets Services, Ltd.,Stepfield, Witham, Essex, UK. Test diets were prepared weekly and stored at room temperature.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups No.	Designation	Dose (ppm)	Animals/sex
1	Control	0	40
3	Low (LDT)	400	40
4	High (HDT)	30,000	40

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day*
Signs of toxicity	All	Twice a day*
Blood samples	10	At 6 weeks
Necropsy	10	At termination (6 weeks)

C. METHODS

1. Observation of blood samples: Blood was collected from the abdominal aorta of anesthetized animals after 6 weeks on the test diets. Observations included the following:

Total tri-iodothyroxine (Total T_3)	Free T_4 index (FT_4I)	Estradiol
Thyroxine	Thyrotrophin	Progesterone
T_4-binding capacity (TBI)	Testosterone	Dehydroepiandrosterone

2. Necropsy The first 10 animals of each sex from each group were anesthetized and blood samples were collected. The livers of each animal were weighed.

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D. STATISTICAL ANALYSIS

1. Continuous variables: (body weight, clinical chemistry, and liver weight.

Statistical procedure	Purpose
Bartlett's Test	Determine homogeneity of variance.*
One-way analysis of variance	Determine significance of variability among all groups.**
Students "t" test	Determine significance of differences between the control and each treatment group.**
Kruskal-Wallis Test	Detect any significant group differences.***

*If variances are not homogeneous ($p>0.05$), the data are transformed (log transformation is used)
**Used on transformed or untransformed data only when variances are shown to be homogeneous or equal.
***Nonparametric test performed on data with heterogenous variances.

II. REPORTED RESULTS

The report stated that there were no mortalities or clinical signs observed in the study. The only effect on body weight noted was a decrease in the weight of females given the 30,000 ppm diet after 6 weeks. The high dose group mean weight was reported to be 197 g, and that for the control group was 274 g. These values were statistically significantly different ($p<0.001$).

There were statistically significant increases in absolute and relative liver weights for both sexes given the 30,000 ppm diet as well as for males given the 400 ppm diet when compared with controls (see Addendum I below).

Addendum II shows group mean clinical chemistry results. The report noted significant increases in thyroxine, free thyroxine index, thyrotrophin, progesterone, and dehydroepiandrosterone in both sexes at 30,000 ppm. The males given the highest dose also showed a significantly increased level of triiodothyroxine. At the 400 ppm dose level, males exhibited a significant increase in thyroxine levels, and females had increased dehydroepiandrosterone levels (see Addendum II below).

ADDENDUM I

Summary (as reported) of Body Weight, Liver Weight, and Liver-to-Body Weight Ratio Results

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CLOFENTEZINE TOXICOLOGY REVIEW

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006783

Reviewed by: Roger Gardner R. G. 5-6-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 6/9/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic feeding study - thyroid function

MRID NUMBER: 404679-09

TEST MATERIAL: Technical grade Clofentezine described as a magenta crystaline powder.

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): TOX/87/167-98; TOX 87293

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT: Technical NC 21314: Investigation into Effects on the Thyroid in the Rat

AUTHOR(S): Mallyon, B. A., and L. P. Markham

REPORT ISSUED: January 5, 1988

DISCUSSION AND CONCLUSIONS: In a 4-week feeding study, groups of 60 male rats were given diets containing 0, 10, 40, 400, or 30,000 ppm clofentezine. There were no effects observed on survival, and no clinical signs or macroscopic pathology were observed in treated animals. Body weight gain was reduced by 15% for the first 3 weeks in the 30,000 ppm dose group, and food consumption for that group was reduced by 3%. The highest dosed group also had increased total protein, globulin, and total cholesterol as well as decreased albumin/globulin ratios. Microsomal UDPGT activity was increased along with liver weight and liver to body and brain weight ratios in the highest dosed group. Thyrotrophin levels were statistically significantly increased, and free tri-iodothyroxine levels were decreased in the 30,000 ppm group males also. The highest dose level was associated with a statistically significant increase in the severity of colloid depletion in the thyroid and a statistically significant increase in the incidence of follicular cell hypertrophy. The incidence of slight to severe focal hypertrophy of the pituitary was also increased in the 30,000 ppm dose group. There was a 10% increase in the liver weight of males given the 400 ppm diet, and microsomal UDPGT activity in those animals was also statistically significantly increased.

Based on these results, a no-observed-effect level (NOEL) was established at 40 ppm, and the lowest-effect level (LEL) was 400 ppm.

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DISCUSSION AND CONCLUSIONS (continued)

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There were adequate data presented in the report to support the conclusions of the investigators.

Core Classification: Supplementary. The study was designed to investigate clofentezine's effect on the thyroid gland of male rats treated for only 4 weeks.

I. PROTOCOL

A. MATERIALS

1. Test compound: The test compound is described as a magenta crystaline substance with unspecified purity.

2. Test species: Male 4-week old Charles River Crl:CD (SD)BR Sprague-Dawley strain rats were used. They weighed from 127 to 190 g at the start of the study. The animals were approximately 6 weeks of age when placed on test diets.

3. Diet preparation: Basal diet consisted of Modified SQC Expanded Rat and Mouse Maintenance Diet No. 1 supplied by Special Diets Services, Ltd., Stepfield, Witham, Essex, UK. Test diets were prepared weekly and stored at room temperature. Samples of test diets were analyzed for stability, homogeneity and accuracy of test concentration before the study was begun. Diets were analyzed for concentration of test substance during the study at unspecified intervals.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

			Animals per group	
No.	Test groups Designation	Dose (ppm)	Clinical chemistry	Histo-pathology
1	Control	0	10	10
2	Low (LDT)	10	10	10
3	Low mid	40	10	10
4	High mid	400	10	10
5	High (HDT)	30,000	10	10

These diets were feed to the test animals for 4 weeks.

Each test group contained 40 additional animals, but only 10 were used for clinical investigations, and 10 were used for histopathological examinations.

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	60	Twice a day*
Signs of toxicity	60	Twice a day*
Body weight	60	At pre-test during randomized group assignment, and at weekly intervals thereafter
Food consumption	60	Weekly**
Blood samples	10	After 4 weeks
Necropsy	10	After 4 weeks

*The report stated that observations were made only once each day on weekends and holidays. The signs, their severity, time of onset, and duration were recorded.
**For each cage of 5 animals.

C. METHODS

1. Observation of blood samples: Blood was collected from animals following decapitation at specified times during the study (see I. B. above).

a. Blood chemistry:

Total protein	Albumin/globulin ratio
Albumin	Total cholesterol
Total globulins	

b. Additional tests: The following tests were also conducted:

Thyrotrophin (TSH)	Total tri-iodothyroxine (TT3)
Total thyroxine (TT4)	Free tri-iodothyroxine (FT3)
Free thyroxine (FT4)	Reverse tri-iodothyroxine (rT3)

2. Other special observations: Microsomal uridine diphosphoglucuronate glucuronyl transferase activity in liver samples prepared at necropsy.

3. Necropsy Gross lesions were noted.

a. Weighed organs: These included the brain and liver.

b. Tissues examined microscopically: The thyroid and pituitary glands were prepared for microscopic examination

The thyroid sections were first examined for histopathology. Then they were recoded and examined again. The report noted that the sections were scored according to the following:

1. Colloid depletion - a reduction in the overall luminal size and staining intensity (degree of eosinophilia) of the colloid.

2. Follicular cell enlargement - cells lining the lumen of the follicle were either squamous in the resting follicles, low cuboidal, cuboidal or columnar in highly active follicles, and lobes with a greater proportion of cuboidal and columnar cells were given a higher score for this condition.

3. Central resting follicles: - islands of follicles were readily distinguished by their large size, eosinophilia of the colloid and squamous appearance of the lining cells from the surrounding, more uniform follicular tissues in some glands.

For the first two conditions, numerical scores were given for these conditions as follows: 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe. The third condition was only rated as present or absent.

The scoring system was also used in examination of pituitary sections. The pituitary lesions were described as follows:

4. Focal hypertrophy - individual and small groups of cells were observed, scattered throughout the anterior lobe, which were enlarged, degranulated and less intensely stained than the surrounding cells.

5. Congestion - the extent to which the sinusoids were engorged with blood.

4. Statistical analyses: According to the report, parametric statistical tests based on the assumption of normally distributed results were used. For results not normally distributed, nonparametric tests such as the Mann Whitney U test were used. Significant differences between treated and control groups were determined by two-tailed procedures, and a probability value of less than 5% ($p \leq 0.05$) was considered statistically significant.

II. REPORTED RESULTS

There were no dose or treatment related signs of toxicity or mortalities in the study according to the investigators.

The highest dosed group showed a 15% decrease in body weight gain for the 4-week feeding period in comparison to the control group value. The highest dosed group also consumed 3% less food than did the control group during the study. These results are summarized as follows:

Observation	Week of Study	Control group	30,000 ppm dose group
Mean body weight (g)	0	153.2	153.6
	1	195.5	188.2*
	2	231.9	220.3*
	3	274.5	250.5*
	4	305.4	287.0*
Body weight gain (g)	0 - 4	152.2	133.4
Mean food consumption (g/animal/day)	1	21	20
	2	22	21
	3	23	23
	4	24	23

*Statistically significant from controls ($p<0.05$; Dunnett's test).

The report stated that the daily test substance intakes for the 10, 40, 400, and 30,000 ppm dose groups were 0.92, 3.73, 37.5, and 2780 mg/kg/day, respectively.

According to the report, the liver-to-body weight ratios for the 400 and 30,000 ppm dose groups were significantly increased above that for the control group. Those results are summarized as follows:

Observation	Control group	400 ppm dose group	30,000 ppm dose group
Terminal body weight (g)	313	299	282**
Brain weight (g)	1.91	1.88	1.91
Liver weight (g)	11.86	12.45	18.11***
Liver to body weight ratio (%)	3.80	4.17***	6.43***
Liver to brain weight ratio (%)	621	652*	951***

*Statistically significantly different from controls ($p<0.05$)
***Statistically significantly different from controls ($p<0.001$)

The authors noted that total protein levels were statistically significantly increased in the 30,000 ppm dose group compared to that of controls, and globulin was statistically significantly increased above the control group value. The globulin increase was reflected in the decreased

II. REPORTED RESULTS (continued)

albumin-to-globulin ratio observed at the highest dose level. Total cholesterol was also significantly increased for the highest dosed group above the control value. These values are summarized as follows:

Observation	Control group	30,000 ppm dose group
Total protein (g/l)	56.4	62.4***
Total globulin (g/l)	28.2	33.2***
A/G ratio	1.0	0.9***
Total cholesterol (mol/l)	1.79	2.65***

**Statistically significantly different from controls ($p<0.01$)
***Statistically significantly different from controls ($p<0.001$)

The 30,000 ppm dose group also showed a statistically significant increase in thyrotropin and a statistically significant decrease in free tri-iodothyroxine. Those results are summarized as follows:

Observation	Control group	30,000 ppm dose group
Thyrotropin (ng/ml)	6.0	12.4***
Free tri-iodothyroxin (umol/l)	2.1	1.7*

*Statistically significantly different from controls ($p<0.05$)
***Statistically significantly different from controls ($p<0.001$)

Liver microsomal UDPGT activity was also increased significantly ($p<0.001$) in the rats from the 30,000 ppm dose group. The control group value was 24 and the high dose group value was 101 (units are expressed as the change in absorbance of nitrophenol per minute per mg microsomal protein). The 400 ppm dosed group also had an increased UDPGT value of 43 which was statistically significantly greater than the control group's value ($p<0.001$).

There were no other differences in clinical chemistry observations that could be associated with treatment according to the investigators.

According to the report, there were no macroscopic observations that were related to treatment.

The report described the histopathology observations as follows:

> At 30000 ppm, slight to severe focal hypertrophy of the pituitary was seen in 7/10 animals. In the thyroid there was a statistically significant increase ($p\leq0.05$) in severity of colloid depletion and moderate to severe hypertrophy of the follicular cells.

The incidences of these lesions as summarized in the original report are presented in the Addendum below.

ADDENDUM

Incidence as Reported of Microscopically
Observed Histopathology in Rats Given Clofentezine
in Their Diet for 4 Weeks

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CLOFENTEZINE TOXICOLOGY REVIEW

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Reviewed by: Roger Gardner R.G. 5-6-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 5/9/88
Section 6, Toxicology Branch (TS 769C)

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic feeding study supplement

MRID NUMBER: 404679-04

TEST MATERIAL: Technical grade Clofentezine (see Addendum)

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): TOX/81/167-22; TOX 81001

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT: Technical NC 21314: 90-Day Dietary Toxicity Study in the Rat (Histopathology review of the thyroid gland) Supplement to Original Report.

AUTHOR(S): Ginocchio, A. V., and I. R. Major

REPORT ISSUED: January 5, 1988

DISCUSSION AND CONCLUSIONS: There were adequate data presented in the supplemental report to indicate that depletion of colloid and follicular cell hypertrophy occurred in male and female rats given diets containing 3000, 9000, or 27,000 ppm for 9 or 13 weeks (see Addendum II below). Those effects were more marked after 13 weeks in comparison to those observed after 9 weeks. These effects were almost completely reversed after four weeks of a recovery period. A no effect level was not established for the thyroid effects in the study.

Core classification: Supplementary. The report is an ammendment to a previously reviewed study.

I. BACKGROUND

This supplemental report describes a re-evaluation of thyroid tissue sections from a 90-day feeding study in rats given diets containing up to 27,000 ppm clofentezine [Ginnocchio, A. V.; and P. N. Brooks. December, 1981. The 90-Day Dietary Toxicity of Pilot Plant Technical NC 21314 (CR 20099/5) to the Rat. Unpublished Report No. TOX/81/167-22 (Study no. 81001) prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.]. The Data Evaluation Record on the 90-day study is included in Addendum I below. The re-evaluation was performed because of the observation that clofentezine caused follicular cell tumors in male rats in a chronic toxicity/oncogenicity study [Ginnocchio, A. V.; and Mallyon, B. A. December 7, 1985. The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofenezine in the Diet to the Rat (Final Report). Unpublished Report No. TOX/84/

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I. BACKGROUND (continued)

167-70 prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.].

II. MATERIALS AND METHODS

The thyroid sections originally stained with hematoxylin and eosin were recoded and examined by another pathologist. The tissue sections were taken from rats given diets containing 0, 3000, 900(or 27,000 ppm clofentezine for nine or 13 weeks. The latter time period also cluded a recovery group (see Addendum I below) .

The report noted that the right and left lobes were examined separately and scored according to the following:

1. Colloid depletion - a reduction in the overall luminal size and staining intensity (degree of eosinophilia) of the colloid.

2. Follicular cell enlargement - cells lining the lumen of the follicle were either squamous in the resting follicles, low cuboidal, cuboidal or columnar in highly active follicles, and lobes with a greater proportion of cuboidal and columnar cells were given a higher score for this condition.

3. Central resting follicles: - islands of follicles were readily distinguished by their large size, eosinophilia of the colloid and squamous appearance of the lining cells from the surrounding, more uniform follicular tissues in some glands.

For the first two conditions, numerical scores were given for these conditions as follows: 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe. The third condition was only rated as present or absent. The investigators stated that the incidence of effects in the right and left lobes were similar, and they were combined in the tabulated results (see Addendum II below).

Results were analyzed by the Kruskal-Wallis Rank test.

III. REPORTED RESULTS

The investigators noted that they observed a clear depletion of colloid and follicular cell hypertrophy in treated male and female rats given diets containing 3000, 9000, or 27,000 ppm for 9 or 13 weeks (see Addendum II below). They also stated that the effects were more marked after 13 weeks in comparison to those observed after 9 weeks. These effects were almost completely reversed after four weeks of a recovery period. A no effect level was not established for the thyroid effects in the study.

ADDENDUM I

Data Evaluation Record for

Ginnocchio, A. V. and P. N. Brooks. December, 1981. The 90-Day Dietary Toxicity of Pilot Plant Technical NC 21314 (CR 20099/5) to the Rat. Unpublished Report No. TOX/81/167-22 (Study no. 81001) prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.

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DATA EVALUATION RECORD
(593A-6)

[redacted]

Citation: Ginocchio, A.W., and P.N. Brooks. January, 1982. The 90-day dietary toxicity of pilot plant Technical NC 21314 (CR 20099/5) to the rat. Unpublished report no. TOX 81/167-22 (study no. 81001) prepared by FBC. Submitted by BFC Chemicals, Inc. EPA Acc. No. 070962.

Materials and Methods

Test substance: Technical NC 21314 (99% purity).

Test species: Male and female Charles River COBS CD Sprague-Dawley rats were used. The animals were approximately 6 weeks old when the experiment began.

Experimental procedure: Rats were assigned to four groups each containing 30 individuals of each sex. Immediately prior to the start of the test, 10 animals of each sex from each group were used for collection of pre-test blood samples. The remaining 20 male and 20 female rats in each group were then given diets containing 0, 3000, 9000, or 27,000 ppm test substance.

The authors noted that results from measurements of total plasma cholesterol at 4 and 6 weeks of the study indicated that a no-observed-effect level (NOEL) would not be established at the dose levels tested. The report also stated that the protocol was modified to evaluate the effects indicated by the elevated total plasma cholesterol levels found in treated rats. The modifications included sacrificing 5 rats of each sex from each group after 64 days of test diet administration. At the end of the 90-day feeding period 10 rats of each sex from each group were sacrificed, while the remaining 5 male and 5 female rats from each group were given control diet for an additional 28-day "regression" period. These modifications were made to evaluate histological changes as well as the reversibility of the effects observed.

All test animals were observed at least once a day for signs of toxicity and mortality. The report stated that observations were made more frequently when necessary, and were not made on weekends or holidays. The signs, their severity, time of onset, and duration were recorded.

Body weights for each animal were obtained on the day of their arrival at the laboratory, at pre-test during randomized group assignment, and at weekly intervals through the feeding and "regression" periods. Animals were also weighed on the day of necropsy. Food and water consumption were determined weekly for each cage of 5 animals.

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Blood samples were taken from 10 male and 10 female rats from each group before the test started. These animals were then discarded from the study. Blood and urine samples were collected from 10 animals of each sex from each group at weeks 4, 6, and 12 of the feeding period and at the end of the 28-day "regression" period. Blood was collected from the retro-orbital sinus, and the animals were fasted overnight prior to sampling.

Hematological observations included hematocrit, hemoglobin, red blood cell and platelet counts, and total and differential white blood cell counts. Blood chemistry observations included total protein, albumin, total globulins, blood urea nitrogen, electrolytes, creatinine, uric acid, glucose, total cholesterol, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates (AP), lactate dehydrogenase (LDH), and triglycerides. Urinalysis included volume, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, specific gravity, and microscopic examination of centrifuged deposits.

Animals were sacrificed after 64 days on test diets (5 per sex per group), at the end of the 90-day feeding period (10 per sex per group), and at the end of the 28-day "regression" period. Animals found in extremis were also sacrificed. These animals and any that were found dead were necropsied. Gross lesions were noted, and the liver, kidneys, spleen, heart, adrenals, gonads, brain, and pituitary were removed and weighed. Tissues of all test rats from the circulatory, respiratory, digestive, endocrine, nervous, excretory, and reproductive systems were prepared for histological examination.

Reported Results

The only clinical sign attributed by the authors to administration of the test substance was loss of hair in treated rats. The authors stated that this observation appeared most frequently during the first 30 days of the experiment, and regressed thereafter. The reported incidence of hair loss during the first 30 days is summarized as follows:

Dose group	Males	Females
Control	0/20	0/20
Low	5/20	10/20
Mid	9/20	10/20
High	16/20	8/20

The only other observed clinical signs were associated with blood sampling according to the report.

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Only two of the 6 deaths reported were associated by the authors with the test substance. These deaths occurred in the high dose group of male rats. One of these male rats died on day 6 while the other died on day 90 of the feeding period. At necropsy the authors noted congestion and hemorrhage in the bladder and prostate of both animals. The bladders were also described as grossly distended. The report stated that one male each from the control, mid, and high dose groups died because of complications from the blood sampling procedure. The sixth death (a male in the mid dose group) was sacrificed in extremis because of a dentition condition.

Body weight decreases were also noted in treated rats when group mean weights were compared with those of controls. The male rats in the high dose group exhibited a 13 to 14% decreased group mean body weight below control males after 8 weeks on the test diet. Group mean body weights for the other treated groups averaged 5 to 8% less than controls during the feeding period for male rats. Group mean body weights for treated female rats averaged 5 to 9% less than controls from the 11th week until the end of the feeding period with the high dose group reported to have a mean body weight decrease of 12% below controls during the 13th week of the study. All groups had comparable mean body weights by the end of the "regression" period according to the reported results.

The only hematological parameters that were effected by administration of the test substance according to the report were hematocrit and hemoglobin measurements at 4 and 12 weeks in the mid and high dose group males and at 12 weeks in the low dose group males. The authors noted that the decreases in hemoglobin and packed cell volume were within the limits observed in rats of the same strain and age in the laboratory, but they also stated that the persistance of the decreases in the mid and high dose groups suggested that the test substance could be the cause of the anemia observed. No other hematological parameters were effected by administration of NC 21314.

Increased levels of plasma cholesterol were reported in treated rats of both sexes. The report stated that these increases were not related to the duration of treatment (progressive), the percentage increase over controls for the 4, 6, and 12 week measurements were summarized as follows:

Dose group	Range of increase (%)
Low	40-80
Mid	70-100
High	80-125

Blood triglyceride levels were also reported to be elevated at 6 weeks (only time measured during dosage administration). Levels in male rats were 14, 38, and 95% higher than controls for the low, mid and high dose groups, respectively. The differences between the mid and high dose groups were statistically significant ($p < 0.05$, Student's t-test). The increases for groups of female rats were 46, 51, and 63% for the low, mid, and high dose groups, respectively.

The authors also noted concurrent increase in plasma protein, albumin, and total globulin which they described as limited but treatment-related. The increases reported did not exceed 8% above values tabulated for control animals.

No other treatment-related effects on clinical chemistry were observed in the study.

The only dose related observation reported from urinalysis results was an orange to reddish brown color which the authors attributed to excretion of the test substance or possible contamination from feces or test diets.

At necropsy the authors stated that the liver was the only organ exhibiting grossly observable effects. These effects included enlargement, darkening of the surface, and accentuated external architecture (the third observation was found in males only). Only the 27,000 ppm group was reported to have these effects. The investigators noted that the surface changes were also seen in the mid dose group males. These liver changes were absent from the 2 males that died during the feeding period (see above), nor were they reported in those animals examined after the "regression" period.

No other gross lesions were found in other organs according to the report.

The organ weights observed by the investigators for the low, mid, and high dose groups averaged 40, 50, and 70% greater than that for controls. Organ-to-body-weight ratios also reflected the increase. Liver weights in rats sacrificed after the 4-week "regression" period were comparable for all treated and control groups.

The only histological observation which the investigators observed to be treatment related was centrilobular hepatocellular enlargement. All of the treated rats observed 9 and 12 weeks were reported to have enlarged centrilobular hepatocytes.

Discussion and Conclusion

There are adequate data in the report to support the conclusion of the authors that the dosages selected for the study were too high to establish a NOEL. However, the study characterizes the primary effects of NC 21314, Those effects indicate that the liver is a target organ for the test substance. Technical NC 21314 increases the liver weight, changes in the gross appearance of the organ, and causes centrilobular hepatocyte enlargement when given to male and female rats at 3000, 9000, or 27,000 ppm in the diet. These effects were shown to be reversible since the liver weight as well as gross and histological changes were not observed in animals examined after the "regression" period.

Clinical chemistry results also reflected liver changes induced by NC 21314. Elevated cholesterol and triglyceride levels observed during the feeding period, dropped after the 4-week "regression" period.

Core Classification: Supplementary. As noted above, the protocol was modified after results indicated to the investigators noted that a NOEl could not be established. The protocol was modified (see Materials and Methods section above) to characterized the effects histologically and determine the reversibility of the effects.

ADDENDUM II

Microscopic Re-examination of Thyroid
Tissue from Male and Female Rats Given Diets
Containing Clofentezine for 90-Days

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CLOFENTEZINE TOXICOLOGY REVIEW

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006783

Reviewed by: Roger Gardner R.G. 7-1-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 7/14/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic feeding study supplement

MRID NUMBER: 404679-05

TEST MATERIAL: Technical grade Clofentezine (see Addendum I below)

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): TOX/81/167-23/1; TOX 81019

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT: The 90-Day Dietary Toxicity Study of Technical NC 21314 (CR 20099/5, Pilot Plant) to the Male and Female Rat (Histopathology review of the thyroid gland). Supplement to the Original Report.

AUTHOR(S): Ginocchio, A. V., and I. R. Major

REPORT ISSUED: January 5, 1988

DISCUSSION AND CONCLUSIONS: There were adequate data presented in the supplemental report to indicate that depletion of colloid and follicular cell hypertrophy occurred in male and female rats given diets containing 400 or 4000 ppm clofentezine for 13 weeks (see Addendum II below). Those effects were more marked in male rats than in female rats. The effects were completely reversed after six weeks of a recovery period. A no effect level was noted for thyroid effects at 40 ppm in female rats.

The lowest effect level was apparently 40 ppm in males. Control group males had minimal to severe colloid depletion and minimal to moderate follicular cell hypertrophy, and the mid dose group males had minimal to slight depletion of colloid and hypertrophy. These effects were shown to be reversible at doses as high as 27,000 ppm (see DER on study MRID No. 404679-04), and they were reversed at 400 ppm by the end of the chronic feeding study. Therefore, the effects observed at the 40 ppm dose level are not toxicologically significant, and 40 ppm can also be described as a NOEL in male rats.

Core Classification: Supplementary. The report is a re-evaluation of tissue sections from a study that was previously reviewed.

I. BACKGROUND

This supplemental report describes a re-evaluation of thyroid tissue sections from a 90-day feeding study in rats given diets containing up 0, 40, 400, or 4000 ppm clofentezine [Ginnocchio, A. V.; and P. N. Brooks. December, 1981. The 90-Day Dietary Toxicity of Pilot Plant Technical NC 21314 (CR 20099/5) to the Rat. Unpublished Report No. TOX/81/167-23/1 (Study no. 81019) prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.]. The Data Evaluation Record on the 90-day study is included in Addendum I below. The re-evaluation was performed because of the observation that clofentezine caused follicular cell tumors in male rats in a chronic toxicity/oncogenicity study [Ginnocchio, A. V.; and Mallyon, B. A. December 17, 1985. The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofentezine in the Diet to the Rat (Final Report). Unpublished Report No. TOX/84/167-70 prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.].

II. MATERIALS AND METHODS

The thyroid sections originally stained with hematoxylin and eosin were recoded and examined by another pathologist. The tissue sections were taken from rats given diets containing 0, 40, 400, or 4000 ppm clofentezine for 13 weeks. Five rats of each sex from each group of 25 were given control diet for 6 weeks following the end of the 13-week feeding period before they were sacrificed. The remaining 20 males and 20 females from each group were sacrificed at the end of the 13-week feeding period.

The report noted that the right and left lobes were examined separately and scored according to the following:

1. Colloid depletion - a reduction in the overall luminal size and staining intensity (degree of eosinophilia) of the colloid.

2. Follicular cell enlargement - cells lining the lumen of the follicle were either squamous in the resting follicles, low cuboidal, cuboidal or columnar in highly active follicles, and lobes with a greater proportion of cuboidal and columnar cells were given a higher score for this condition.

3. Central resting follicles: - islands of follicles were readily distinguished by their large size, eosinophilia of the colloid and squamous appearance of the lining cells from the surrounding, more uniform follicular tissues in some glands.

For the first two conditions, numerical scores were given for these conditions as follows: 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe. The third condition was only rated as present or absent. The investigators stated that the incidence of effects in the right and left lobes were similar, and they were combined in the tabulated results (see Addendum II below).

Results were analyzed by the Kruskal-Wallis Rank test.

III. REPORTED RESULTS

The report described the results as follows:

Colloid depletion :
In males, 12/19 at 4000 ppm and 5/20 at 400 ppm showed the condition to at least a moderate extent, whereas 3/20 controls and 2/19 at the low dose showed this degree of thyroid activity. A mild but appreciable depletion of colloid was produced in a few female rats at 4000 and 400 ppm...

In those animals that had been withdrawn from compound administration...at the end of the experiment the thyroid glands of all treated animals could not be distinguished from the controls.

Follicular cell size :
In males, the effect was moderate at 4000 ppm and slight at 400 ppm. A few females showed a slight response at the two higher treatment levels. Among the recovery animals, thyroid glands of treated rats could not be distinguished from those of control rats.

Central resting follicles :
These were present in 7/20 males receiving 400 ppm of the compound compared with only 1/20 in the controls, but there were none at all at the high dose and the complete absence of a dose response relationship indicates that this is not of any biological significance.

Results as tabulated in the original report are included in Addendum II below.

ADDENDUM I

Data Evaluation Record for

Ginnocchio. A. V.· and P. N. Brooks. December. 1981. The 90-Day Dietary Toxicity of Pilot Plant Technical NC 21314 (CR 20099/5) to the Rat. Unpublished Report No. TOX/81/167-23/1 (Study no. 81019) prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.]

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DATA EVALUATION RECORD
(593A-7)

006783

Citation: Ginocchio, A.W., and P.N. Brooks. December, 1981. The 90-day dietary toxicity of pilot plant Technical NC 21314 (CR 20099/5) to the rat. Unpublished report No. TOX 81/167-23/1 (study no. 81019) prepared by FBC. Submitted by BFC Chemicals, Inc. EPA Acc. No. 070963.

Materials and Methods

Test substance: Technical NC 21314 (99% purity)

Test species: Male and female Charles River COBS CD Sprague-Dawley rats were used. The animals were approximately 6 weeks old when the experiment began.

Experimental procedure: Rats were assigned to four groups each containing 35 individuals of each sex. Immediately prior to the start of the test, 10 animals of each sex from each group were used for collection of pre-test blood samples. The remaining 25 male and 25 female rats in each group were then given diets containing 0, 40, 400, or 4000 ppm test substance for 13 weeks. Five rats of each sex were continued on control diets for an additional 6-week "regression" period for an evaluation of reversibility of the effects observed.

All test animals were observed at least once a day for signs of toxicity and mortality. The report stated that observations were made more frequently when necessary, and were not made on weekends or holidays. The signs, their severity, time of onset, and duration were recorded.

Body weights for each animal were obtained on the day of their survival at the laboratory, at pre-test during randomized group assignment, and at weekly intervals through the feeding and "regression" periods. Animals were also weighed on the day of necropsy. Food and water consumption were determined weekly for each cage of 5 animals.

Blood samples were taken from 10 male and 10 female rats from each group before the test started. These animals were then discarded from the study. Blood and urine samples were collected from 10 animals of each sex from each group at weeks 4, 8, and 12 of the feeding period and at the end of the 6-week "regression" period. Blood was collected from the retro-orbital sinus, and the animals were fasted overnight prior to sampling.

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Hematological observations included hematocrit, hemoglobin, red blood cell and platelet counts, and total and differential white blood cell counts. Blood chemistry observations included total protein, albumin, total globulins, blood urea nitrogen, electrolytes, creatinine, uric acid, glucose, total cholesterol, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), and triglycerides. Urinalysis included volume, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, specific gravity, and microscopic examination of centrifuged deposits.

Animals were sacrificed after 13 weeks on test diets (20 per sex per group) and at the end of the 6-week "regression" period (5 per sex per group). Animals found in extremis were also sacrificed. These animals and any that were found dead were necropsied. Gross lesions were noted, and the liver, kidneys, spleen, heart, adrenals, gonads, brain, and pituitary were removed and weighed. Tissues of all test rats from the circulatory, respiratory, digestive, endocrine, nervous, excretory, and reproductive systems were prepared for histological examination.

Reported Results

None of the animals died during the study.

Body weight differences were no more than 5 (high dose male rats) to 8% (high dose female rats) less than control group mean body weights at the end of the feeding period, and all groups had comparable mean body weights at the end of the "regression" period according to the report. The report also stated that the food consumption was also decreased by the same amount. Female rats from the high dose groups were also reported to have consumed 5 to 10% more water than the controls during the feeding period.

Although reported hemoglobin concentrations for some treated groups appeared to be decreased from control values, the decreases were only as high as 7% at 12 weeks for the high dose females. Other decreases in the 400 or 4000 ppm groups were from 3 to 5%. No other changes in hematological parameters were noted.

The treatment-related changes in clinical chemistry noted by the authors were increased cholesterol and increased total blood protein. In the 400 ppm group of male rats cholesterol levels were increased above control values by 13 to 18% at weeks 4, 8, and 12, while those values for the 4000 ppm male rats were increased by 24 to 50% above control males. The increases noted

for female rats in the 400 ppm group ranged from 23% at week 8 to 50% at week 12. In the 4000 ppm group females the cholesterol levels ranged from 51% to 102% at weeks 8 and 12, respectively.

Increases in total protein were noted in the report, and the increases in the 400 and 4000 ppm groups ranged from 3 to 9% above control values at 4, 8, or 12 weeks.

There were no consistent dose-related effects reported on the other clinical chemistry parameters examined.

The only dose-related effect observed in urinalysis was coloration by the test substance. The authors stated that the coloration could have resulted from excretion of NC 21314 or contamination of urine samples by treated diets or feces.

Organ weight increases were noted in the liver in treated groups when compared with control liver weights. Liver weights in male and female rats in the high dose group were approximately 40% greater than those in the control group, while those in the mid dose group were approximately 10% greater.

Other organs weight increases were noted in the spleen and kidneys, but these increases were not consistent or associated with dose.

No gross lesions that could be associated with treatment were seen at necropsy according to the report.

The only histological change noted by the authors was centrilobular hepatocyte enlargement (CLHE). The incidences of this lesion were 7, 13, and 20 of 20 in the low, mid, and high dose group males, respectively. In the female rats, the incidences were 0 of 20 in the low and mid dose groups and 20 of 20 in the high dose group. No control rats or the rats examined at the end of the "regression" period exhibited CLHE according to the report. The lesions were classified as slight, minimal, moderate, or marked. Those observed in the low dose group male rats were described as localized and slight.

Discussion and Conclusions

There were adequate data to support the authors conclusions that the 400 and 4000 ppm diets caused elevated cholesterol levels and increases in liver weights and liver-to-body-weight ratios. However, a no-observed-effect-level (NOEL) for centrilobular hepatocellular enlargement was not established. The microscopic lesions have been re-evaluated (see DATA EVALUATION RECORDS 593A-8, and -9).

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Core Classification: Supplementary because of the lack of a NOEL for CLHE in male rats. This classification can be upgradable provided the re-evaluation mentioned above can establish that the results in the low dose group males are not toxicologically significant.

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ADDENDUM II

Microscopic Re-examination of Thyroid
Tissue from Male and Female Rats Given Diets
Containing Clofentezine for 90-Days

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CLOFENTEZINE TOXICOLOGY REVIEW

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006783

Reviewed by: Roger Gardner R.G. 5-6-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 5/9/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic feeding study supplement

MRID NUMBER: 404679-06

TEST MATERIAL: Technical grade Clofentezine (see Addendum I below)

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): TOX/84/167-70; TOX 82003

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Schering Agrochemicals Ltd.

TITLE OF REPORT:

AUTHOR(S): Ginocchio, A. V., and I. R. Major

REPORT ISSUED: January 5, 1988

DISCUSSION AND CONCLUSIONS: There were adequate data presented in the supplemental report to indicate that depletion of colloid, follicular cell hypertrophy, and agglomeration of colloid occurred in male rats given a diet containing 400 ppm clofentezine for 12 months (see Addendum II below). Those effects were diminished in those rats after 27 months. No thyroid effects were evident in treated female rats or in male rats given diets containing 10 or 40 ppm clofentezine during the study. The no-observed-effect level (NOEL) for the thyroid gland effects was 40 ppm, and the lowest-observed effect level (LOEL) was 400 ppm (highest dose tested).

The results also confirmed the incidence of thyroid tumors originally reported (see Addenda I and II below).

Core Classification: Supplementary. The report is a re-evaluation of tissue sections from a study that was previously reviewed.

I. BACKGROUND

This supplemental report describes a re-evaluation of thyroid tissue sections from a chronic feeding study in rats given diets containing 0, 10, 40, or 400 ppm clofentezine [Ginnocchio, A. V.; and Mallyon, B. A. December 17, 1985. The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofentezine in the Diet to the Rat (Final Report). Unpublished Report No. TOX/84/167-70 prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.]. The Data Evaluation Record on the study is included in Addendum I below.

II. MATERIALS AND METHODS

The thyroid sections originally stained with hematoxylin and eosin were recoded and examined by another pathologist. The tissue sections were taken from rats given diets containing 0, 10, 40, or 400, ppm clofentezine for 27 months (see Addendum I below for additional information).

The report noted that the thyroid sections were scored according to the following:

1. Colloid depletion - a reduction in the overall luminal size and staining intensity (degree of eosinophilia) of the colloid.

2. Follicular cell enlargement - cells lining the lumen of the follicle were either squamous in the resting follicles, low cuboidal, cuboidal or columnar in highly active follicles, and lobes with a greater proportion of cuboidal and columnar cells were given a higher score for this condition.

3. Central resting follicles: - islands of follicles were readily distinguished by their large size, eosinophilia of the colloid and squamous appearance of the lining cells from the surrounding, more uniform follicular tissues in some glands.

4. Agglomeration of the colloid - the colloid in the follicular lumen appeared to have condensed into clumps which were sometimes single, but often multiple. These clumps or agglomerates were surrounded by eosinophilic hyaline material, somewhat paler than the normal colloid. Not all follicles were affected and the number varied between animals.

5. Mineralization of colloid - single or multiple, intensely basophilic bodies appeared in the lumen of some follicles. Selected specimens were positive by the Von Kosa and Alizarin Red S methods for calcium. It is possible that these represented agglomerates of some standing which had undergone mineralization or formed independently.

6. Follicular cystic hyperplasia - a focus comprised of a few follicles with greatly enlarged lumen lined by one or two layers of low cuboidal epithelium which may possess hyperchromatic nuclei. There may be some destruction of the follicular walls and they may appear to form papillary projections, although these are probably the abbreviated remnants of the follicular wall. Adjacent follicles are not compressed and there is no sign of encapsulation.

7. Follicular cell adenoma - a circumscribed area of follicular tissue either totally or partially encapsulated or causing compression of adjacent non-neoplastic tissue. The number of follicular epithelial cells is significantly higher than in normal follicles and their nuclei are hyperchromatic. There is usually much more variation in follicle size than in either cystic hyperplasia or normal thyroid tissue.

II. MATERIALS AND METHODS (continued)

8. Follicular cell carcinoma - an area of tissue arising from follicular epithelium of an invasive nature, overgrowing a fibrous capsule, growing in solid sheets of cells with some undergoing mitosis or with areas of bizarre nuclear morphology.

For conditions 1, 2, 4, and 5, numerical scores were given as follows: 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe, and 5 = very severe. The other non-neoplastic conditions were rated as present or absent. The investigators stated that the incidence of effects in the right and left lobes were similar, and they were combined in the tabulated results (see Addendum II below).

Results were analyzed by the Kruskal-Wallis Rank test.

III. REPORTED RESULTS

Addendum II below contains a description of the results and tabulated incidences as reported.

The investigators concluded that the 400 ppm level caused a marked colloid depletion, mild follicular cell hypertrophy, and moderate agglomeration of colloid in the thyroid gland of male rats after 12 months of treatment. These effects appeared to diminish after 27 months of treatment. The incidence of thyroid tumors was also increased at the highest dose tested, and 5 of the 8 animals observed with tumors had malignant follicular cell tumors.

There were no thyroid effects observed in treated female rats or in male rats given the 10 and 40 ppm dose levels.

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ADDENDUM I

Data Evaluation Record for

Ginnocchio, A. V.; and Mallyon, B. A. December 17, 1985. The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofentezine in the Diet to the Rat (Final Report). Unpublished Report No. TOX/84/167-70 prepared by Huntingdon Research Centre and FBC Limited. Submitted by Nor-Am Cehmical Co. EPA Acc. No. 262268.

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R.H 8-25-87

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Reviewed by: Roger Gardner
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. JH 5/25/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic feeding/Oncogenicity (Guideline §83-5)

ACCESSION NUMBER: 262261, 262262

TEST MATERIAL: Technical grade Clofentezine (CR 20099/12, purity 98.7%) was used. It was described as a magenta colored crystaline substance.

SYNONYMS: Clofentezine; NC 21314; APOLLO®; 3,6-bis(2chlorophenyl)-1,2,4, 5-tetrazine

STUDY NUMBER(S): TOX/ 84/167-70

SPONSOR: FBC Limited; Nor-Am Cehmical Co.

TESTING FACILITY: Huntingdon Research Centre

TITLE OF REPORT: The Oncogenicity and Chronic Toxicity of Technical NC 21314 (Chlofentezine in the Diet to the Rat (Final Report).

AUTHOR(S): Ginnocchio, A. V.; and Mallyon, B. A.

REPORT ISSUED: December 17, 1985

CONCLUSIONS: Clofentezine was administered in the diet to male and female Charles River Crl:CD Sprague-Dawley BR strain rats for up to 27 months at levels of 0, 10, 40, or 400 ppm. Clofentezine treatment was associated with a slight increase in free thyroxine levels in blood taken from males at 27 months. Liver weights and weight ratios for high dose group males were increased above those values for controls (by approximately 20%) and for high dose group females (by approximately 10%). Those group differences were statistically significant. Histological changes occurred in the liver of males in a dose-related manner, and they included centrilobular hepatocyte hypertrophy and vacuolation, focal cystic degeneration of hepatocytes, and diffuse distribution of fat deposits in the livers of high dose group males.

Based on these observations, a no-effect level was established in the experiment at 40 ppm (2 mg/kg/day), and the lowest-effect level was 400 ppm (20 mg/kg/day).

The incidence of mammary tumors in female rats decreased with dose, and according to the report, the incidence of thyroid follicular cell tumors in male rats exhibited a statistically significant positive dose-related trend ($p<0.05$) primarily because of the highest dose group's higher incidence in comparison to that in the control group. Reported incidences were adjusted by censoring those animals that died before the first diagnosis was made (week 88), and a

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CONCLUSIONS (continued)

Fisher's Exact test on the control and high dose group incidences showed the difference between the groups was statistically significant ($p = 0.024$). The adjusted results also showed a significant positive trend ($p < 0.005$; Cochran-Armitage test).

Core classification: Minimum

I. PROTOCOL

A. MATERIALS

1. Test compound: The test compound is described as a magenta crystaline substance with unspecified purity of 98.7%.

2. Test species: Male and female 4-week old Charles River Crl:CD Sprague-Dawley BR strain rats were used. The males weighed from 57 to 79 g, and the females weighed from 53 to 78 g on receipt at the laboratory. The animals were approximately 6 weeks of age when placed on test diets.

3. Diet preparation: Basal diet consisted of Modified SQC Expanded Rat and Mouse Maintenance Diet No. 1 supplied by Special Diets Services, Ltd.,Stepfield, Witham, Essex, UK. Test diets were prepared weekly and stored at room temperature. Samples of test diets were analyzed for stability, homogeneity and accuracy of test concentration before the study was begun. Diets were analyzed for concentration of test substance during weeks 1, 2, 3, 4, 8, 11, 15, 16, 18, 22, 25, 29, 30, 33, 37, 41, 45, 49, 53, 57, 61, 65, 69, 73, 77, 81, 85, 89, 93, 97, 101, 105, 108, 112, 116, and 120.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups		Dose	Animals per sex		
No.	Designation	(ppm)	Main study*	Pre-test	Interim Sacrifice**
1	Control	0	50	10	20
2	Low (LDT)	10	50	10	20
3	Mid	40	50	10	20
4	High (HDT)	400	50	10	20

*30 months.
**At 12 months

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day*
Signs of toxicity	All	Twice a day*
Body weight	All	On day of arrival at laboratory, at pre-test during randomized group assignment, at weekly intervals through the first 13 weeks, biweekly thereafter, and on the day of necropsy.
Food consumption	All	Weekly**
Water consumption	All	Weekly**
Ophthalmology	50†	At 12 months
Blood samples	10***	Pre-test
	10	At 6, 12, 18, and 27 months.
Urine samples		
Necropsy	Animals found dead or moribund	When found.
	20†	At 12 months
	50†	At 27 months

*The report stated that observations were made only once each day on weekends and holidays. The signs, their severity, time of onset, and duration were recorded.
**For each cage of 5 animals.
***These animals were discarded from the study.
†All survivors in the main study group only.

C. METHODS

1. Observation of blood samples: Blood was collected from the retro-orbital sinus, and the animals were fasted overnight prior to sampling.

a. Hematology

X	Hematocrit	X	Differential white cell counts
X	Hemoglobin	X	Mean corpuscular hemoglobin concentration
X	Red cell count		
X	Platelet count	X	Mean cell volume
X	Total white cell count	X	Mean corpuscular hemoglobin

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C. METHODS (continued)

1. Observation of blood samples (continued)

b. Blood chemistry

X Total protein	X Uric acid	X Alkaline phosphatase (AP)
X Albumin	X Glucose	X Lactate dehydrogenase (LDH)
X Total globulins	X Total cholesterol	X Triglycerides
X Albumin/globulin ratio	X Total bilirubin	
X Blood urea nitrogen	X Aspartate aminotransferase (AST)	
X Electrolytes	X Alanine aminotransferase (ALT)	
X Creatinine		

c. Additional tests: The following tests were conducted after the final sacrifice (27 months).

Total tri-iodothyroxine (Total T_3)	T_4-binding capacity (TBI)	Testosterone
Thyroxine	Free T_4 index (FT_4I)	Estradiol
	Thyrotrophin	Progesterone

2. Urine observations

X Volume	X glucose	X occult blood	X specific gravity
X pH	X ketones	X urobilinogen	X microscopic examination of centrifuged deposits
X protein	X bilirubin		

3. Necropsy Gross lesions were noted.

a. Weighed organs

X Liver	X Spleen	X Adrenals	X Brain
X Kidneys	X Heart	X Gonads	X Pituitary

b. Tissues examined microscopically

X Adrenals	X Liver	X Kidneys
X Aorta	X Lungs	X Spleen
X Bone and marrow	X Lymph nodes	X Spinal cord
X Brain	X Mammary glands	X Stomach
X Cecum	X Esophagus	X Testes/ovaries
X Colon	X Pancreas	X Thymus
X Duodenum	X Pituitary	X Thyroid with parathyroid
X Ears	X Prostate	
X Epididymides	X Rectum	X Trachea
X Eyes with Hardarian gland	X Salivary gland	X Urinary bladder
	X Seminal vessicles	X Uterus with cervix
X Heart	X Sciatic nerve	X Vagina
X Ileum	X Skeletal muscle	X All macroscopic abnormalities
X Jejunum	X Skin and subcutis	

D. STATISTICAL ANALYSIS

1. Continuous variables: (body weight, hematology, clinical chemistry, organ and weights.

Statistical procedure	Purpose
Bartlett's Test	Determine homogeneity of variance.*
One-way analysis of variance	Determine significance of variability among all groups.**
Students "t" test	Determine significance of differences between the control and each treatment group.**
Kruskal-Wallis Test	Detect any significant group differences.***

*If variances are not homogeneous ($p>0.05$), the data are transformed (log transformation is used)
**Used on transformed or untransformed data only when variances are shown to be homogeneous or equal.
***Nonparametric test performed on data with heterogenous variances.

2. Frequency data (gross and microscopic observations at necropsy)

( ) One-tailed or (X) two-tailed probability values were calculated.

Statistical procedure	Purpose
isher's Exact Test hi-square Tests with Yates' correction	Determine significance of differences between individual groups, overall variations, or trends
ruskal-Wallis Test	Determine significance of differences for graded responses.

3. Other methods: (data adjustments or transformations, criteria of significance, etc)

II. REPORTED RESULTS 006232

An 18-month interim report (Ginnocchio and Mallyon, 1983) on this study was reviewed previously (see Addendum I below). Therefore, the results described in this section are primarily from observations made during the 18th through 27th months of the study.

A. Mortality and Signs of Toxicity: No treatment-related effects were observed according to the report. Mortality during the last 9 months of the study is summarized as follows:

Dose (ppm)	Mortalities* during weeks Males 78-104	Males 104-120	Females 78-104	Females 104-120
0	13	8	19	6
10	15	7	18	9
40	13	4	14	7
400	16	3	18	4

*Excludes those animals sacrificed at 12 months and during weeks 52 through 77.

Soiling of the eyes and nose, hair loss, and abrasions were the most frequently observed clinical signs, but the report stated that these occurred at a low incidence which was unrelated to treatment.

B. Body Weight, Food and Water Consumption: These observations in treated groups were described as comparable to those in the control groups throughout the study.

C. Test Substance Intake: The average daily intake for treated animals was reported as follows:

Dose (ppm)	Daily dose (mg/kg/day) Males	Females	Both sexes
10	0.43	0.55	0.49
40	1.72	2.18	1.95
400	17.3	22.1	19.7

D. Clinical Pathology

1. Hematology: The investigators noted that there were sporadic statistically significant differences between treated and control groups, but the differences were not dose-related, and they were within normal ranges. A decrease in hemoglobin and mean cell hemoglobin concentration was noted for the 400 ppm females when compared to those values in the control females. The group mean hemoglobin values for the control and high dose group females were 14.9 and 14.1 g/dl, respectively ($p<0.01$) at 18 months. The respective group means at 27 months were 15.8 and 13.8 g/dl. The mean cell hemoglobin concentrations at 18 months were 19.9 pg in the control group and 19.7 pg for the high dose group (no statistically significant difference). At 27

1. Hematology (continued): 006232

months the control and high dose group means were 15.8 and 14.8 pg, respectively ($p<0.05$).

2. Clinical chemistry: The only significant difference noted by the authors at 27 months was in the group mean thyroxine level of 400 ppm males in comparison to that of control group males. The group mean for the control group was reported as 13.4 pmol/l while that for the 400 ppm dose group was 19.7 pmol/l ($P<0.05$).

Although the investigators noted other statistically significant differences for clinical chemistry parameters, these were described as unrelated to dose or not occurring consistently during the course of the experiment.

3. Urinalysis: The only observation the authors described as unusual was discoloration of the urine from female rats given the 400 ppm diet. The urine from these animals appeared bright orange, and the color was attributed to the test substance or its metabolites in the urine.

E. Ophthalmology: There were no treatment-related effects observed according to the report.

F. Necropsy

1. Organ weights: The only treatment related effect on organ weight was observed for the liver. Group mean values for those animals sacrificed at 27 months are summarized as follows:

	Dose level (ppm)			
	Males		Females	
Observation	0	400	0	400
Body weight (g)	563	589	415	430
Liver weight (g)	13.74	17.05†	10.12	11.37*
Liver/body weight ratio (%)	2.44	2.90 **	2.45	2.66**

*Statistically significantly different from controls ($p<0.05$)
**Statistically significantly different from controls ($p<0.01$)
†Statistically significantly different from controls ($p<0.001$)

2. Gross observations: There were no gross observations reported at necropsy of animals after the interim or terminal sacrifice that the investigators could associate with treatment.

3. Histopathology:

a. Non-neoplastic observations

i) General observations: Several lesions were observed to occur in statistically significant patterns such as trends, apparently treatment related effects, or a difference between one treated group and an appropriate control group (see Tables 1a and b for examples). However, most of these observations

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a. Non-neoplastic observations (continued)

were not associated by the investigators with the test substance because trends were not observed consistently at the interim and terminal sacrifices or in animals that died during the experiment, and trends and differences were not significant when overall incidences (total of all animals) were analyzed.

Table 1a

Summary of non-neoplastic lesions reported to exhibit statistically significant differences between groups or a dose-related trend in male rats.

Observation	Dose level (ppm) 0	10	40	400
Epididymis:				
Reduced spermatozoa				
Intercurrent deaths†††	5/26	4/26	13/23**	8/29
Kidney:				
Chronic interstitial nephrosis				
Interim sacrifice†	0/20	3/20	0/20	4/20
Hydronephrosis				
Interim sacrifice	3/20	2/20	1/20	1/20
Intercurrent deaths††	4/26	7/26	2/23	1/29
Terminal sacrifice	1/24	3/24	3/27	1/31
Overall	8/70	10/70	6/70	2/70
Pyelitis				
Overall****	0/70	3/70	8/70	2/70
Increased fat deposition (cortical tubules				
Intercurrent deaths††	3/26	3/26	1/23	0/29

*Statistically significant negative dose-related trend ($p<0.01$).
**Statistically significantly different from controls ($p<0.05$).
***Overall group variation statistically significant ($p<0.05$).
****Overall group variation statistically significant ($p<0.01$).
†Statistically significant positive dose-related trend ($p<0/05$).
††Statistically significant negative dose-related trend ($p<0.05$).

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Table 1b

Summary of non-neoplastic lesions reported to exhibit statistically significant differences between groups or a dose-related trend in female rats.

Observation	Dose level (ppm) 0	10	40	400
Adrenal medulla:				
Focal hyperplasia				
Interim sacrifice	0/20	0/20	0/20	0/20
Intercurrent deaths†	1/25	5/31	3/28	0/27
Terminal sacrifice	5/20	4/19	5/20	5/23
Overall	6/65	9/70	8/68	5/70
Kidney:				
Glomerular tubular nephropathy				
Interim sacrifice	1/20	3/20	0/20	0/20
Intercurrent deaths††	6/30	7/31	7/29	14/27*
Terminal sacrifice	6/20	7/19	8/29	8/23
Overall	6/70	7/70	8/69	22/70
Pelvic mineralization				
Interim sacrifice	6/20	5/20	6/20	8/20
Intercurrent deaths	23/30	15/31	18/29	17/27
Terminal sacrifice†††	12/20	7/19	14/20	20/23
Overall	41/70	27/70	38/69	45/70
Increased fat deposition (cortical tubules)				
Intercurrent deaths††	1/30	2/31	5/29	6/27
Liver:				
Foci/areas of telangiactasis				
Intercurrent deaths††	2/30	3/31	3/30	6/27
Terminal sacrifice††	10/20	9/19	11/20	16/23
Parathyroid:				
Hyperplasia				
Terminal sacrifice	3/20	8/18*	8/17*	2/22
Overall	3/65	8/60*	8/58*	2/66

†Statistically significant negative dose-related trend ($p<0.01$).
††Statistically significant positive dose-related trend ($p<0/05$).
†††Statistically significant positive dose-related trend ($p<0/01$).
*Statistically significantly different from controls ($p<0.05$).

a. Non-neoplastic observations (continued)

Hardarian gland: The investigators noted that a subclinical epidemic of a sialodacryoadenitis virus infection was present in test animals at the time of the interim sacrifice and affected the test groups unevenly (see Addendum below for additional information). An increased incidence of male rats with mononuclear cell infiltration of the hardarian gland in the 400 ppm group observed at that time was attributed to the infection since animals dying in the latter portion of the study as well as animals sacrificed at termination of the study did not exhibit the effect.

Spleen: The degree of pigment deposition was affected in a statistically significant manner (a positive dose-related trends in female rats). The incidence of those lesions is summarized as follows:

Observation	Dose level (ppm) 0	10	40	400
Interim sacrifice				
Minimal	5/20	2/20	0/20	0/20
Slight	6/20	11/20	4/20	6/20
Moderate	8/20	7/20	11/20	11/20
Marked	1/20	0/20	5/20	3/20
Intercurrent deaths*				
Minimal	7/30	10/31	5/30	2/27
Slight	10/30	9/31	9/30	10/27
Moderate	10/30	12/31	12/30	13/27
Marked	3/30	0/31	3/30	2/27
Terminal sacrifice				
Minimal	10/20	0/19	14/20	0/23
Slight	9/20	9/19	3/20	7/23
Moderate	1/20	9/19	2/20	12/23
Marked	0/20	1/19	1/20	4/23

*Statistically significant positive dose-related trend when severity of lesion is considered ($p<0.01$).

**Statistically significant positive dose-related trend when severity of lesion is considered ($p<0.05$).

When sections were stained with Perl's stain and examined, only a slightly significant ($p<0.1$) increase in pigment deposition was noted in females at the interim sacrifice. The reported incidences are as follows:

Observation	Dose level (ppm) 0	10	40	400
Minimal	0/20	0/20	1/20	0/20
Slight	1/20	0/20	2/20	1/20
Moderate	13/20	11/20	6/20	6/20
Marked	6/20	8/20	10/20	12/20
Severe	0/20	1/20	0/20	1/20

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a. Non-neoplastic observations (continued)

The report indicated that these lesions were unlikely to be associated with treatment.

ii) Organs affected by test substance

Liver: Males exhibited dose-related effects, and incidences of some of some of the most frequently observed lesions are summarized in Table 2 below. According to the report female rats were not affected in a manner that could be associated with administration of the test substance.

The incidence of male rats with focal cystic hepatocyte degeneration was not statistically significantly different for treatment groups when compared with controls, and a significant dose-related trend ($p<0.05$) was found only when severity of the lesion was considered for animals dying during the study. The incidence of cystic hepatocyte degeneration in those animals is summarized as follows:

Observation	Dose level (ppm) 0	10	40	400
Number examined	26	26	23	29
Focal cystic degeneration of hepatocytes* (minimal)	5	4	2	9
(slight)	2	1	1	1
(moderate)	1	0	0	0

*Statistically significant trend when severity of lesion was taken into account.

a. Non-neoplastic observations

ii) Organs affected by test substance (continued)

Table 2

Summary of non-neoplastic lesions reported to exhibit statistically significant differences between groups or a dose-related trend in the liver of male rats.

Observation	Dose level (ppm) 0	10	40	400
Fat deposits in non-specific distribution				
Intercurrent deaths	4/26	1/26	3/23	8/29
Terminal sacrifice	7/24	5/24	6/27	11/21
Total†	11/70	6/70	9/70	19/70
Groups of finely vacuolated hepatocytes				
Terminal sacrifice††	10/24	11/24	8/27	15/21
Prominant areas of vacuolated hepatocytes				
Intercurrent deaths	0/26	0/26	0/27	4/29
Terminal sacrifice	1/24	7/24	1/27	3/21
Overall***	1/70	7/70	1/70	7/70
Centrilobular hepatocyte enlargement				
Interim sacrifice	0/20	0/20	0/20	18/20*
Intercurrent deaths	1/26	0/26	0/23	3/29
Terminal sacrifice	0/24	0/24	0/27	10/21*
Overall	1/70	0/70	0/70	31/70*
Centrilobular hepatocyte vacuolation				
Interim sacrifice†	3/20	6/20	1/20	10/20**
Intercurrent deaths	0/26	2/26	1/23	0/29
Terminal sacrifice	0/24	0/24	0/27	1/21
Overall†	3/70	8/70	2/70	11/70**
Focal hepatocyte necrosis				
Interim sacrifice†	2/20	0/20	0/20	5/20
Intercurrent deaths	3/26	1/26	0/23	3/29
Terminal sacrifice	0/24	1/24	1/27	0/21
Overall	5/70	2/70	1/70	8/70

*Statistically significantly different form controls ($p<0.001$).
**Statistically significantly different form controls ($p<0.001$).
***Statistically significant between groups variation ($p<0.05$).
†Statistically significant positive dose-related trend ($p<0.01$)/
††Statistically significant positive dose-related trend ($p<0.05$).

a. Non-neoplastic observations 006232

ii) Organs affected by test substance (continued)

Thyroid: The incidence of agglomeration of colloid in males was affected in a dose-related manner at terminal sacrifice. The incidence of that effect is summarized as follows:

Observation	Dose level (ppm) 0	10	40	400
Interim sacrifice				
Minimal	7/20	3/20	5/20	4/19
Slight	1/20	1/20	1/20	3/19
Moderate	1/20	4/20	2/20	6/19
Marked	1/20	0/20	0/20	1/19
Total*	10/20	8/20	8/20	14/20
Intercurrent deaths				
Minimal	4/26	1/21	1/20	6/22
Slight	9/26	1/21	3/20	6/22
Moderate	4/26	0/21	1/20	1/22
Marked	0/26	0/21	0/20	0/22
Total	17/26	2/21	5/20	8/22
Terminal sacrifice				
Minimal	4/24	4/23	9/27	4/21
Slight	6/24	5/23	4/27	11/21
Moderate	2/24	1/23	1/27	3/21
Marked	0/24	0/23	1/27	0/21
Total**	12/24	10/23	15/27	18/21***

*Statistically significant positive dose-related trend ($p<0/01$).
**Statistically significant positive dose-related trend ($p<0.001$).
***Statistically significantly different from controls ($p<0.05$)

b. Neoplastic lesions

Adrenal medulla: The authors noted that 8 of the 12 pheochromocytomas found in female rats were observed in the 10 ppm dose group. The incidence of pheochromocytomas are summarized as follows:

Observation	Dose level (ppm) 0	10	40	400
Pheochromocytomas				
Interim sacrifice	0/20	0/20	0/20	0/20
Intercurrent deaths				
Benign	0/25	5/31	0/23	1/27
Malignant	0/25	0/31	2/28	0/27
Terminal sacrifice				
Benign	0/20	3/19	1/20	0/23
Malignant	0/20	0/19	0/20	0/23
Overall*	0/65	8/70**	1/68	1/70

*Statisticallly significant variation between groups ($p<0.01$).
**Statistically significantly different from controls ($p<0.05$).

These tumors were not considered to be related to the administration of the test substance because they did not occur in a dose-related manner.

b. Neoplastic lesions (continued)

Mammary tumors: The incidence of mammary tumors in female rats exhibited a statistically significant negative dose-related trend. Incidences of those tumors were reported as follows:

Observation	Dose level (ppm) 0	10	40	400
Fibroadenomas (one or more/animal)				
Interim sacrifice	0/20	1/20	3/20	0/20
Intercurrent deaths	13/30	12/31	10/30	6/27
Terminal sacrifice	1/20	2/19	2/20	2/23
Overall	14/70	15/70	15/70	8/70
Adenomas (one or more/animal)				
Intercurrent deaths	3/30	5/31	2/30	0/27
Terminal sacrifice	1/20	0/19	1/20	1/23
Overall	4/70	5/70	3/70	1/70
Adenocarcinomas (one or more/animal)				
Intercurrent deaths	2/30	4/31	4/30	2/27
Terminal sacrifice	4/20	1/19	4/20	2/23
Overall	6/70	5/70	8/70	4/70
Mammary tumors combined*				
Grand total†	28/70	23/70	21/70	16/70**

*Includes animals with one or more mammary tumors of any type.
**Statistically significantly different from controls ($p<0.05$).
†Statistically significant negative dose-related trend ($p<0.05$).

Thyroid: The investigators noted a statistically significant positive trend with respect to the incidence of follicular cell tumors in males ($p<0.05$). The incidence of the tumor in males was reported as follows:

b. Neoplastic lesions (continued)

Observation	Dose level (ppm) 0	10	40	400
Follicular cell hyperplasia				
Interim sacrifice	0/20	0/20	2/20	1/20
Intercurrent deaths	1/26	0/26	1/23	1/29
Terminal sacrifice	1/24	2/24	5/27	3/21
Follicular cell tumor				
Interim sacrifice				
Benign	0/20	0/20	0/20	0/20
Probably malignant	0/20	0/20	0/20	0/20
Malignant	0/20	0/20	0/20	0/20
Total	0/20	0/20	0/20	0/20
Intercurrent deaths				
Benign	1/26	0/26	0/23	0/29
Probably malignant	0/26	0/26	0/23	0/29
Malignant	1/26	0/26	1/23	0/29
Total	2/26	0/26	1/23	0/29
Terminal sacrifice				
Benign	0/24	1/24	0/27	3/21
Probably malignant	0/24	0/24	0/27	2/21
Malignant	0/24	1/24	1/27	3/21
Total*	0/24	2/24	1/27	8/21
Overall				
Benign	1/70	1/70	0/70	3/70
Probably malignant	0/70	0/70	0/70	2/70
Malignant	1/70	1/70	1/70	3/70
Total*	2/70	2/70	1/70	8/70

*Statistically significant positive dose-related trend ($p<0.05$).

III. DISCUSSION

A. Authors' conclusions: According to the authors, there were no marked or persistant treatment-related effects on behavior or condition, body weight, food and water consumption, hematology, urine analysis, or ophthalmological observations.

Effects the investigators associated with the test substance included a slight increase in free thyroxine levels in blood taken from males at 27 months.

Liver weights and weight ratios for male and female rats given the 400 ppm diet were slightly elevated above that for control group animals, but no statistically significant increase in absolute liver weights were noted in those groups.

III. DISCUSSION (continued)

Histological changes occurred in the liver and thyroid of males in a dose-related manner, and they included agglomeration of colloid in the thyroid, centrilobular hepatocyte enlargement and vacuolation, focal cystic degeneration of hepatocytes, and diffuse distribution of fat deposits in the livers of high dose group males. Based on these observations, the investigators concluded that a no-effect level was established in the experiment at 40 ppm (1.95 mg/kg/day).

The authors noted that the incidence of mammary tumors in female rats decreased with dose, and the incidence of thyroid follicular cell tumors in male rats exhibited a statistically significant positive dose-related trend (primarily because of the highest dose).

B. Reviewer's Conclusions:

1. Non-neoplastic effects: There were adequate data presented to support the author's conclusions that clofentezine had no effects on behavior or condition, body weight, food and water consumption, hematology, urine analysis, or ophthalmological observations. Based on the liver and thyroid effects, a noobserved-effect level of 40 ppm and a lowest-effect level of 400 ppm are established by the experiment.

2. Neoplastic effects:

a. Thyroid tumors: The first follicular cell tumor was observed in a control group male that died during week 88 of the study. The other thyroid tumor in that group was found during week 114. The only other follicular cell tumor observed during the study was in one male of the mid dose group at week 114. The remainder of the follicular cell tumors were observed in rats sacrificed at the end of the study, including all eight tumors observed in the high dose group.

If reported incidences are adjusted by censoring those animals sacrificed at 12 months and those dying before the first diagnosis was made in the control group (week 88), the incidences would be 2 of 47, 2 of 41, 2 of 40, and 8 of 40 for the control, low, mid, and high dose groups, respectively. A Fisher's Exact test on the control and high dose group incidences shows that the difference between the groups is statistically significant ($p = 0.024$). The adjusted results also show a significant positive trend ($p < 0.005$; Cochran-Armitage test).

There was no statistically significant increase in the incidence of follicular cell hyperplasia (see page 16 above), and no follicular cell tumors were noted in those animals with hyperplasia.

b. Other neoplastic lesions: There were adequate data presented in the report to support the conclusions of the authors. Administration of Clofentezine in the diet of male and female rats for over two years did not increase the incidence of tumors other than those observed in the thyroid of treated males.

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IV. References

Ginnocchio, A. V., and B. A. Mallyon. December, 1983. The Oncogenicity and Chronic Toxicity of Technical NC 21 314 (Clofentezine) in the Diet of the Rat. Interim Report: At 18 Months) Unpublished report prepared by FBC Limited. Report No. TOX/ 83/167-62. Submitted by BFC Chemicals, Inc. Wilmington, DE. EPA Acc. No. 257993.

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ADDENDUM

Data Evaluation Record of the Interim Report

innocchio, A. V., and B. A. Mallyon. December, 1983. The Oncogenicity and hronic Toxicity of Technical NC 21 314 (Clofentezine) in the Diet of the Rat. nterim Report: At 18 Months) Unpublished report prepared by FBC Limited. eport No. TOX/ 83/167-62. Submitted by BFC Chemicals, Inc. Wilmington, DE. PA Acc. No. 257993.

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DATA EVALUATION REPORT

1. CHEMICAL: Clofentezine; NC 21314; APOLLO®, 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine

2. TEST MATERIAL: Technical grade Clofentezine (CF 20099/12, purity unspecified) was used.

3. STUDY/ACTION TYPE: Chronic - rats (Interim Report)

4. STUDY IDENTIFICATION: Ginnocchio, A. V., and E. A. Mellyon. December, 1983. The Oncogenicity and Chronic Toxicity of Technical NC 21 314 (Clofentezine) in the Diet of the Rat. Interim Report: At 18 Months) Unpublished report prepared by FBC Limited. Report No. TOX/ 83/167-62. Submitted by BFC Chemicals, Inc. Wilmington, DE. EPA Acc. No. 257953.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
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Signature: Roger Gardner
Date: 11-28-85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 11 8/28/85

7. CONCLUSION: Results from an interim sacrifice of 20 rats of each sex from each group suggest that a no-observed-effect level (NOEL) for liver effects (slightly increased absolute and relative weight and hepatocellular hypertrophy) is 40 ppm in the diet. Test diets were fed for 12 months, and the effects were more evident in male rats than in females. The lowest-effect level (LEL) was 400 ppm (highest dose tested).

Core classification: Supplementary. The report is an 18-month interim report on a long-term feeding study. Many of the effects observed are present in the group of animals that were sacrificed at 12 months (islet cell hyperplasia in the pancreas and elevated serum glucose) or in those continued on test diets (an increase in mortality during the 13th through the 18th months of the study). These effects do not consistently appear in both groups, and they are not consistently statistically significant. Final conclusions regarding the toxicological significance of those results can not be made until the final report is made available.

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B. MATERIALS AND METHODS

Test species: Male and female 4-week old Charles River Crl:CD Sprague-Dawley FF strain rats were used. The males weighed from 57 to 75 g, and the females weighed from 53 to 71 g on receipt at the laboratory. The animals were approximately 6 weeks of age when placed on test diets.

Experimental procedure: Rats were assigned to four groups each containing 80 individuals of each sex. Immediately prior to the start of the test, 10 animals of each sex from each group were used for collection of pre-test blood samples. The remaining 70 male and 70 female rats in each group were then given diets containing 0, 10, 40, or 400 ppm test substance for up to 18 months.

All test animals were observed twice a day for signs of toxicity and mortality. The report stated that observations were made only once each day on weekends and holidays. The signs, their severity, time of onset, and duration were recorded.

Body weights for each animal were obtained on the day of their arrival at the laboratory, at pre-test during randomized group assignment, at weekly intervals through the first 13 weeks of the study, and biweekly thereafter. Animals were also weighed on the day of necropsy. Food and water consumption were determined weekly for each cage of 5 animals.

As mentioned above, blood samples were taken from 10 male and 10 female rats from each group before the test started. These animals were then discarded from the study. During the feeding period, blood and urine samples were collected from 10 animals of each sex from each group at weeks 25, 51, and 77. Blood was collected from the retro-orbital sinus, and the animals were fasted overnight prior to sampling.

Hematological observations included hematocrit, hemoglobin, red blood cell and platelet counts, and total and differential white blood cell counts. Indices calculated from some of these observations included mean corpuscular hemoglobin concentration, mean cell volume, and mean corpuscular hemoglobin. Blood chemistry observations included total protein, albumin, total globulins, albumin/globulin ratio, blood urea nitrogen, electrolytes, creatinine, uric acid, glucose, total cholesterol, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), and triglycerides. Urinalysis included volume, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, specific gravity, and microscopic examination of centrifuged deposits.

Ophthalmological examinations were conducted at 12 months on all surviving rats with the exception of those scheduled for sacrifice at that time.

Twenty animals of each sex were sacrificed after 12 months on test diets. Animals found in extremis during the feeding period were also sacrificed. These rats and any that were found dead were necropsied; gross lesions were noted; and the liver, kidneys, spleen, heart, adrenals, gonads, brain, and

6. MATERIALS AND METHODS (continued)

pituitary were removed and weighed. The following tissues were processed for microscopic examination:

Adrenals	Heart	Rectum	Thymus
Aorta	Ileum	Salivary gland	Thyroid with parathyroid
Bone and marrow	Jejunum	Seminal vesicles	Trachea
Brain	Liver	Sciatic nerve	Urinary bladder
Cecum	Lungs	Skeletal muscle	Uterus with cervix
Colon	Lymph nodes	Skin and subcutis	Vagina
Duodenum	Mammary glands	Kidneys	All macroscopic abnormalities
Ears	Esophagus	Spleen	
Epididymides	Pancreas	Spinal cord	
Eyes with Harderian gland	Pituitary	Stomach	
	Prostate	Testes/ovaries	

9 REPORTED RESULTS

The report stated that there were no treatment-related effects on mortality (see Table 1 and Section 10. DISCUSSION). There were also no treatment-related effects on body weight, food or water consumption, behavior, condition, or occurrence of clinical signs according to the report. No consistent or dose-related effects on the hematological, clinical chemistry (with the exception of phosphate, sodium, and glucose levels discussed below), or urine analysis parameters monitored during the study. Isolated statistically significant differences between treatment groups and the controls with respect to those parameters were described as within normal limits for the age and strain of the test animals.

Table 1

Mortality in the main study

Dose (ppm)	Mortalities* during weeks: Males 1-26	Males 27-52	Males 52-78	Females 1-26	Females 27-52	Females 52-78
0	0	3	0	0	1	4
25	1	0	3	0	0	4
50	1	1	5	1	0	3
100	1	1	1	1	1	1

*Excludes those animals sacrificed at 12 months.

The investigators reported a statistically significant increase in relative liver weight for high dose group rats (see Table 2). The increase was associated with an increased incidence of centrilobular hepatocyte hypertrophy in males (see Table 3).

9. [illegible] (continued)

Table 2

Group mean body and liver weights and weight ratios for rats sacrificed at 12 months

Parameter	Males		Females	
	0 ppm	400 ppm	0 ppm	401 ppm
Body weight (g)	609	601	340	332
Liver weight (g)	15.89	17.68	8.16	8.45
Liver/body weight ratio (%)	2.61	2.94**	2.41	2.56*

*Statistically significantly different ($p<0.05$)

**Statistically significantly different ($p<0.01$)

Other effects noted by the investigators included an increased group mean serum glucose and decreased phosphate and sodium levels for the high dose males (see Table 4). There was also an increased incidence of islet cell hyperplasia in the high dose group males above controls (see Table 3 and section 10. DISCUSSION below).

The tumor incidence observed during the first 18 months of the study is summarized in Tables 5 and 6. There was no dose-related incidence observed according to the authors. The first tumor was diagnosed in a mid-dose group female that died during week 18 of the study; it was a lymphosarcoma according to the report. The earliest tumor observed in male rats was found in a high-dose group animal that died during week 44 of the study. The tumor was also reported to be a lymphosarcoma.

11. [illegible]

Although the authors concluded that there were no effects on mortality, Table 1 (page 5, above) suggests that there might be a dose-related trend in males during the last half of the study. Another trend, which did not appear to be statistically significant, was the increased incidence of islet cell hyperplasia (Table 3) in male rats sacrificed at 12 months (Chi square = 1.251, $p = 0.2631$ for high dose group males when compared with controls). In addition, the effects on serum glucose levels in males is apparently associated with dose, but significant differences between treated and control group males noted during the first half of the study disappear during the last half (at 77 weeks). This pattern of response emphasizes the need for the final report before conclusions can be made regarding mortality, pancreatic effects, and serum glucose results.

Table 5

Most frequent histopathological observations (non-neoplastic) in animals sacrificed after 12 months

Observation	Dose level (ppm) 0	10	40	400
Males				
Liver				
Number examined	20	20	20	20
Focal vacuolation	3	3	8	5
Centrilobular hepatocyte vacuolation	3	6	1	10*
Centrilobular hepatocyte hypertrophy	0	0	0	18**
Bile duct proliferation	1	1	2	3
Focal hepatocyte necrosis	2	2	0	5
Pancreas				
Number examined	20	18	19	19
Pigment deposits	7	3	8	12
Islet cell hyperplasia	5	4	6	9
Harderian gland				
Number examined	20	20	20	20
Interstitial mononuclear cells	3	2	5	10*
Females				
Liver				
Number examined				
Centrilobular hepatocyte hypertrophy	0	0	2	2
Focal hepatocyte necrosis	2	2	1	5

*Statistically significant difference from controls ($p<0.05$)

**Statistically significant difference from controls ($p<0.001$)

Table 4

Selected group mean clinical chemistry results for male rats

Dose (ppm)	Phosphate (mmol/l) Pre-test	Week 25	Week 51	Week 77	Sodium (mmol/l) Pre-test	Week 25	Week 51	Week 77	Glucose (mmol/l) Pre-test	Week 25	Week 51	Week 77
0	3.70	2.56	2.64	2.02	145.6	150.0	155.3	144.8	3.4	6.2	6.5	6.2
10	3.76	2.32	2.27**	2.02	144.2	144.2†	151.6	142.2**	3.2*	7.3	6.2	7.1
40	4.02*	2.54	2.46	2.00	145.2	145.5**	151.3	144.4	3.7	6.8	7.1**	6.6
400	4.00*	2.15†	2.20†	1.92	143.4**	142.2†	145.1**	144.6	3.4	7.5**	7.5**	6.7

*Statistically significantly different from controls ($p<0.05$)

**Statistically significantly different from controls ($p<0.01$)

†Statistically significantly different from controls ($p<0.001$)

Table 5

Incidence of tumors (number of animals with a tumor/20 animals) in rats sacrificed at 12 months

Observation	Dose level (ppm) 0	10	40	400
Males				
Pituitary, pars anterior adenoma	0	2	1	0
Thyroid, follicular cell adenoma	0	1	0	0
Subcutaneous fibroma	0	1	0	0
Squamous papilloma	0	0	1	0
Thymus, squamous cell carcinoma and fibrosarcoma	0	1	0	0
Females				
Pituitary, pars anterior adenoma	3	2	2	2

Table 6

Incidence of tumors (number of animals with a tumor/number bearing tumors) in rats dying during the first 18 months (as reported in Table 17b of the original report)

Observation	Dose level (ppm) 0	10	40	400
Males				
Myxofibroma	1/3	0/0	0/1	0/1
Fibrosarcoma	1/3	0/1	0/1	0/1
Leiomyosarcoma	1/3	0/0	0/1	1/1
Lymphosarcoma	1/3	0/0	0/1	1/1
Females				
Lymphosarcoma	0/1	0/0	1/1	0/1

11. DISCUSSION (continued)

On the other hand, elevated absolute and relative liver weights were also slightly increased (see Table 2) for the high dose group males. These weight changes and the increased incidence of hepatocellular hypertrophy were consistent with results from subchronic studies (Cinnochio and Brooks, 1981 and 1982; Brooks and Turnbull, 1983, and FBC Limited, 1983). Therefore, a no-observed-effect level (NOEL) on the basis of liver effects is established at 40 ppm, and the lowest observed effect level (LEL) is 400 ppm (highest dose tested).

Since there were no deaths during the first year of the study among the animals scheduled for the 12-month interim sacrifice and the diffences between the mid and low-dose groups were not significant, the NOEL's and LEL's for the other effects are likely to be the same as those for the liver effects.

12. REFERENCES

Cinnochio, A. V., and F. N. Brooks. December, 1981. The 90-day dietary toxicity of pilot plant technical NC 21314 (CR 20099/5) to the rat. Unpublished report no. TOX/81/167B/1 prepared by FBC Limited. Submitted by EPC Chemicals, Inc. EPA Acc. No. 070694. Doc. 1 Page 16

Cinnochio, A. V., and F. N. Brooks. January, 1982. The 90-day dietary toxicity of technical NC 21314 (CR 20099/5) to the rat. Unpublished report no. TOX/82/167-22 prepared by FBC Limited. Submitted by EPC Chemicals, Inc. EPA Acc. No. 070983. Doc. 1 Page 11

Brooks, F. N., and C. J. Turnbull. January 26, 1983. Technical NC 21 314: 90-Day toxicity study in the rat--Additional examination of the liver histology. Unpublished report no. TOX/83/167-11 prepared by FBC Limited. Submitted by EPC Chemicals, Inc. EPA Acc. No. 071384. Doc. 1 Page 20

FBC Limited. June, 1983. Histopathological report on livers from FBC Study No. TOX 81019 report titled NC 21314: 90-day dietary toxicity study in the rat. Unpublished report prepared by Huntingdon Research Centre for FBC Limited. Submitted by EPC Chemicals, Inc. EPA Acc. No. 071859.

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ADDENDUM II

Microscopic Re-examination of Thyroid
Tissue from Male and Female Rats Given Diets
Containing Clofentezine for 90-Days

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CLOFENTEZINE TOXICOLOGY REVIEW

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___ Description of the product manufacturing process

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Reviewed by: Roger Gardner R.G. 5-6-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 5/9/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic feeding study supplement

MRID NUMBER: 404679-10

TEST MATERIAL: Technical grade Clofentezine (see DER for MRID No. 404679-06)

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

STUDY NUMBER(S): TOX 82003

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: Ohio State University, Department of Veterinary Pathology

TITLE OF REPORT: Histopathologic Evaluation of Thyroid and Parathyroid Glands from Male and Female Sprague-Dawley Rats Fed Clofentezine and Benazolin-Ethyl.

AUTHOR(S): Capen, C.

REPORT ISSUED: September 1, 1987

DISCUSSION AND CONCLUSIONS: One original diagnosis of a benign follicular cell tumor in the high dose group was changed to a diagnois of follicular cell adenocarcinoma in the re-evaluation.

Core Classification: Supplementary. The report is a re-evaluation of slides from the rat chronic feeding study.

I. BACKGROUND

This supplemental report describes a second re-evaluation of thyroid tissue sections from the chronic feeding study in rats. The study has been discussed in a Data Evaluation Record (DER) for the report on the first re-evaluation of those tissues (study MRID No. 404679-06). The DER for the clofentezine chronic study is included as an Addendum to the DER for study MRID 404679-06.

This supplemental report also contains a re-evaluation of thyroid sections from another study which was used to provide historical control data for the clofentezine results.

II. MATERIALS AND METHODS

The thyroid sections originally stained with hematoxylin and eosin were re-examined by a third pathologist. The tissue sections were taken from rats given diets containing 0, 10, 40, or 400 ppm clofentezine for 27

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II. MATERIALS AND METHODS (continued)

months (see Addendum I of the DER for MRID No. 404679-06 for more information).

The report noted that thyroid lesions were diagnosed according to criteria included in Addendum I below.

III. REPORTED RESULTS

The incidence of progressive thyroid lesions as reported in the re-evaluation of the clofentezine chronic feeding study was reported as follows:

Observation	Dose level (ppm) 0	10	40	400
Follicular cell hyperplasia (cystic and/or diffuse)				
Interim sacrifice	0/20	1/20	2/20	1/19
Intercurrent deaths	2/26	0/26	0/23	2/29
Terminal sacrifice	3/24	2/24	8/27	3/21
Total	5/70	3/70	10/70	6/69
Follicular cell tumor				
Interim sacrifice				
Benign	0/20	0/20	0/20	0/19
Malignant	0/20	0/20	0/20	0/19
Total	0/20	0/20	0/20	0/19
Intercurrent deaths				
Benign	0/26	0/26	1/23	0/29
Malignant	2/26	0/26	0/23	0/29
Total	2/26	0/26	1/23	0/29
Terminal sacrifice				
Benign	0/24	1/24	0/27	2/21
Malignant	0/24	1/24	1/27	6/21
Total*	0/24	2/24	1/27	8/21
Overall				
Benign	0/70	1/70	1/70	2/69
Malignant	2/70	1/70	1/70	6/69
Total*	2/70	2/70	2/70	8/69

*Statistically significant positive dose-related trend ($p<0.05$).

III. REPORTED RESULTS (continued)

Statistically significant differences and trends for thyroid tumors in male rats were reported as follows:

Observation	Dose level (ppm) 0	10	40	400
Follicular cell carcinomas				
Intercurrent deaths	2/26	0/26	0/23	0/29
Terminal sacrifice	0/24†††	1/24	1/27	6/21*
Total	2/50†	1/50	1/50	6/50
Follicular cell tumors (any)				
Intercurrent deaths	2/26	0/26	1/23	0/29
Terminal sacrifice	0/24†††	2/24	1/27	8/21**
Total	2/50††	2/50	2/50	8/50

*Statistically significantly different from controls ($p<0.05$: Chi^2 test).
**Statistically significantly different from controls ($p<0.01$: Chi^2 test).
†Statistically significant trend ($p<0.05$; Chi^2 Trend Analysis).
††Statistically significant trend ($p<0.01$; Chi^2 Trend Analysis).
†††Statistically significant trend ($p<0.001$; Chi^2 Trend Analysis).

IV. DISCUSSION

The incidence of follicular hyperplasia (diffuse and/or cystic) reported in the re-evaluation did not appear to be significantly increased by clofentezine in the diet of male rats at levels of 0, 10, 40, or 400 ppm for up to 27 months. There were no statistically significant differences between any of the treated groups and the control group as indicated by Fisher's Exact Test (p values were all > 0.138), and there was no dose response indicated by in incidence of hyperplasia.

One original diagnosis of a benign follicular cell tumor in the high dose group was changed to a diagnois of follicular cell adenocarcinoma in the re-evaluation.

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ADDENDUM

Criteria for Microscopic Re-examination of Thyroid Tissue from Male and Female Rats Given Diets Containing Clofentezine for 27-Months

CLOFENTEZINE TOXICOLOGY REVIEW

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APPENDIX III

Data Evaluation Record
Describing Relevant Data for Peer Review Committee's
Reconsideration of the Oncogenic Potential of Clofentezine

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006783

Reviewed by: Roger Gardner R.G. 7-13-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W Hauswirth 7/13/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

Peer Review of Additional Data
on Clofentezine

CHEMICAL: Clofentezine CASWELL NUMBER: 593AA

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine

CONCLUSIONS: Because of the increased incidence of thyroid tumors in male rats in the chronic feeding study, re-evaluations of thyroid slides and additional studies of clofentezine's effects on the thyroid were conducted.

Since two experiments demonstrated that clofentezine does not selectively accumulate in the thyroid gland, and iodine uptake is increased in the gland, an indirect effect was investigated. The studies considered below showed that short-term administration of high doses of clofentezine (3000 to 30,000 ppm in the diet) caused increased liver enzyme activity and increased bile flow in male rats. Results of two subchronic experiments at 30,000 ppm suggested that these effects changed the metabolism and excretion pattern for thyroid hormone, but the studies did not indicate that these changes would be biologically significant when a lower dose level (400 ppm) is administered chronically.

Enhanced clearance of thyroid hormones could reduce blood levels of thyroxine sufficiently to cause the pituitary gland to secrete thyroid stimulating hormone (TSH) to increase thyroid gland activity. In male rats given diets containing 30,000 ppm clofentezine for 6 weeks, elevated TSH levels were observed, but thyroxine levels were also increased. In addition, the thyroxine levels were elevated in male rats at the end of the chronic study without an increase in their TSH levels. No thyroid blood chemistry tests were conducted during earlier portions of the chronic feeding study, and the 400 ppm dose level in a 6 week feeding study did not significantly increase thyroxine or TSH levels. These circumstances suggest that a longer study over a broader dose range would more clearly characterize the biological significance of clofentezine's effects on the thyroid in male rats.

Microscopic observation of thyroids from rats in chronic and subchronic studies indicated increased activity in the glands of treated male rats. There was a dose-related increase in the severity of colloid depletion along with dose-related increases in the incidence and severity of follicular cell hypertrophy, and an increased incidence of hyperplasia. In short-term studies at high doses (3,000 to 30,000 ppm), follicular cell hypertrophy was reversed in the three weeks following a 9-week treatment period. Observation of hyperplasia was limited to the chronic feeding study, and its incidence was not dose-related. Thyroid weights were increased along with these microscopic changes but not in a statistically significant manner. These marginal changes further emphasize the need for a chronic study using a broader dose range to more clearly support the suggested indirect mechanism for clofentezine's oncogenic potential.

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I. Background

A. First Toxicology Branch Peer Review

On September 16, 1987, the Toxicology Branch Peer Review Committee considered available data on clofentezine in a weight-of-the-evidence analysis and classified the oncogenic potential of the chemical into Category C (possible human oncogen) based on the following:

1. Clofentezine was associated with an increase in tumors (benign and malignant combined, see Table 1 below) in only one of two species tested, and the tumors occurred in one sex of a single strain of the rat.

2. The observed response exceeded the historical control range, and was observed at the highest dose tested which was well below the Maximum Tolerated Dose (MTD) predicted by subchronic feeding studies.

3. Clofentezine was not mutagenic.

4. The chemical is not structurally related to known carcinogens.

In addition, the available information on specific thyroid effects considered by the Committee did not indicate that the induction of thyroid tumors was related to inhibition of thyroid function by clofentezine treatment in the rat.

A quantitative risk assessment was conducted based on the adjusted incidences of combined benign and malignant thyroid tumors in male rats (see Table 1) from the 27-month feeding study. The unit risk in human equivalents (multistage model Q_1^*) based on those data is 0.053 $(mg/kg/day)^{-1}$.

B. Scientific Advisory Panel Review

On March 2, 1988, the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) considered the Agency's weight-of-the-evidence considerations and classification of the oncogenic potential of clofentezine in a public meeting. Additional data provided by the Registrant and discussed below was also considered by the Panel.

The Panel commented on the biological significance of follicular cell tumors in male rats given clofentezine as follows:

> Thyroid tumors in rats may or may not be biologically significant for humans. If data clearly demonstrate increases in TSH (thyroid stimulating hormone) and the subsequent responses in the thyroid (hyperplasia and neoplasia), this endpoint does not provide evidence of direct, compound-induced carcinogenicity. However, if such data do not exist for other chemicals, thyroid tumors should be considered as evidence for carcinogenic potential.

Table 1

Incidence[+] of Follicular Cell Tumors in the Thyroid of Male Rats Receiving Clofentezine in the Diets for up to 27 Months (1).

Tumor type	Dose (ppm) 0	10	40	400
Benign only	1/68 (1)*	1/65 (2)	0/66 (0)	3/63 (5)
Malignant only	1/68 (1)*	1/65 (2)	2/66 (3)	5/63 (8)
Combined benign and malignant	2/68 (3)**	2/65 (3)	2/66 (3)	8/63 (13)*

[+]Number of tumor bearing animals/number of animals at risk (and percentage). Animals that died before week 52 have been excluded.
*P < 0.05 **P < 0.01
Note: Significance of trend (determined by Cochran-Armitage test) denoted at the control group value, and significance of pairwise comparison (determined by Fisher's Exact test) denoted at dose group value.

The Panel agreed with the Peer Review determinations that there were no compound-related effects on tumor incidence in the mouse oncogenicity study and that clofentezine is not mutagenic. However they did not believe that failure to achieve a Maximum Tolerated Dose (MTD) compromises the rat study or that the thyroid tumors in male rats provide evidence of human risk for carcinogenicity. Based on these considerations, the Panel concluded:

> ...(clofentezine) belongs in category D. This interpretation is based primarily on the data demonstrating increased TSH, hyperplasia, and decreased half-life of T_4 and T_3. It is well known that this sequence leads to thyroid tumors in rats. Exposure to agents that cause this sequence in rats has not resulted in increased TSH, hyperplasia, and thyroid tumors in humans. Therefore, there is inadequate data for suggesting human carcinogenicity.

The Panel then concluded that the data were not adequate for a quantitative risk assessment.

C. Proposed Mechanism for Thyroid Tumor Induction

In rats, clofentezine is expected to increase liver enzyme activity and bile flow causing an increase in the metabolism and excretion of thyroxine. This increased turnover reduces the level of circulating thyroxine. The pituitary, through the hypothalamus, responds to reduced thyroxine blood

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levels by secreting thyroid stimulating hormone (TSH). Elevated TSH levels then stimulate follicular cells of the thyroid gland to replenish depleted blood levels of the hormone in clofentezine treated rats. This mechanism is proposed as the cause of increased thyroxine blood levels, colloid depletion, follicular cell hypertrophy, hyperplasia, and neoplasia observed in male rats at the end of the chronic feeding study.

Data presented at the SAP meeting in support of the proposed mechanism for follicular cell carcinogenesis observed in the chronic feeding study are discussed in the sections that follow according to criteria proposed by Hill, et al. (1987). Those criteria are described as follows:

1. Goitrogenic activity in vivo (i. e., thyroid follicular cell hypertrophy, and hyperplasia).

2. Clinical chemistry indication of changes in thyroid and pituitary functional parameters (e. g., reduced thyroid hormone and increased TSH serum concentrations).

3. Specific evidence that the agent either reduces thyroid hormone synthesis (e. g., inhibits iodine uptake) or increases thyroid hormone clearance (e. g., enhances biliary excretion).

4. A progression of lesions under long-term exposure to an agent, showing cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia (benign and possibly malignant tumors).

5. Other studies bearing on the hypothesis that thyroid-pituitary imbalance may be operative, like reversibility of lesions following cesation of the treatment.

6. Structure activity analysis of the agent under review to see if it belongs to a class of compounds that shows a correlation with the induction of thyroid tumors.

II. Discussion

A. Goitrogenic Activity

The incidences of hypertrophy, hyperplasia, and neoplasia observed in the rat chronic feeding study are summarized in Table 2. below.

In the original report and one re-evaluation of thyroid gland slides follicular cell hypertrophy and hyperplasia were rated according to the following criteria:

> ...cells lining the lumen of the follicle were either squamous in the resting follicles, low cuboidal, cuboidal or columnar in highly active follicles, and lobes with a greater proportion of cuboidal and columnar cells were given a higher score for this condition.

...a focus comprised of a few follicles with greatly enlarged lumen lined by one or two layers of low cuboidal epithelium which may possess hyperchromatic nuclei. There may be some destruction of the follicular walls and they may appear to form papillary projections, although these are probably the abbreviated remnants of the follicular wall. Adjacent follicles are not compressed and there is no sign of encapsulation.

Hypertrophy was scored in the first re-evaluation on a scale from 0 (minimum) to 5 (very severe), and follicular cell hyperplasia was rated as present or absent without a grade.

Table 2

Summary of Microscopic Lesions Observed
in Male Rats Fed Clofentezine for up to 27 Months.

Observation	Dose level (ppm) 0	10	40	400
Follicular cell hypertrophy				
Original Report **	4/69	3/65	3/67	5/63
Re-evaluation 1 **	16/70	19/67	26/67	32/67
Re-evaluation 2 ***	-	-	-	-
Follicular cell hyperplasia				
Originally reported	2/70	2/70	8/70	5/70
Re-evaluation 1 **	3/70	2/67	3/68	7/69
Re-evaluation 2	5/70	3/70	10/70	6/69
Follicular cell tumor				
Overall				
Benign	1/70	1/70	0/70	3/70
Probably malignant	0/70	0/70	0/70	2/70
Malignant	1/70	1/70	1/70	3/70
Total*	2/70	2/70	1/70	8/70

*Statistically significant positive dose-related trend ($p<0.05$; Cochran-Armitage trend test).
**Follicular cell size was not assessable in some animals according to these reports, and the lesion was graded as follows: 1 = minimum, 2 = slight, 3 = moderate, 4 = severe.
***Not assessed in this re-evaluation.

Thyroid weights were not statistically significantly affected (see Table 3 below), but the group mean weights for the organ were greater in treated male rats than in the control group.

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Table 3

Summary of Group Mean Thyroid Weights (g) and Organ-to-Body-Weight Ratios (%) in Clofentezine Treated Male Rats

Observation	Dose level (ppm) 0	10	40	400
	At 12 months			
Body weight	609	585	615	601
Thyroid weight	0.027	0.026	0.024	0.026
Thyroid weight ratio	0.005	0.004	0.004	0.004
	At 27 months			
Body weight	563	623	630**	589
Thyroid weight	0.035	0.045	0.044	0.050
Thyroid weight ratio	0.006	0.007	0.007	0.008

**Statiscitally significantly different from controls (pn0.01; Fisher's Exact Test).

The observation of hypertrophy in the chronic feeding study indicated dose-related increases in incidence and severity (see Table 2), but observations of hyperplasia did not clearly suggest a dose-related increase in that lesion. In addition, thyroid weights were not clearly increased over the dose range tested. These results only suggest that clofentezine has a goitrogenic effect associated with its toxicity.

B. Clinical Chemistry Indications

Thyroid blood chemistry was evaluated at the end of the chronic feeding study in rats, and in a short-term study in which rats received diets containing as much as 30,000 ppm clofentezine for up to 6 weeks. Tables 4 and 5 summarize the results from these studies.

At the end of the chronic feeding study, the amount of free thyroxine was statistically significantly increased in male rats given the 400 ppm diet. There were no increased TSH levels noted in any of the three treatment groups (see Table 4 below).

The short-term feeding study indicated that thyroxine was statistically significantly increased by clofentezine in the diet at 400 and 30,000 ppm. The 30,000 ppm dose level was also associated with statistically significantly increased tri-iodothyroxine and thyrotrophin (TSH) blood levels. These results are not consistent with expected changes since an elevated TSH level is usually found with a decreased thyroxine level.

Table 4

Group Mean Values (and Standard Deviations) for Thyroid Blood Chemistry Tests in Male Rats Treated with Clofentezine for 27 Months.

Observation	Dose level (ppm) 0	10	40	400
Total tri-iodothyroxine (nmol/l)	2.0 (0.6)	2.1 (0.5)	1.9 (0.3)	1.9 (0.3)
Thyroxine (nmol/l)	34 (8)	32 (5)	34 (6)	35 (6)
Thyroxine binding capacity	0.54 (0.06)	0.55 (0.11)	0.61 † (0.10)	0.57 (0.08)
Free thyroxine index *	63 (14)	65 (29)	67 (12)	62 (13)
Free tri-iodothyroxine (pmol/l)	1.4 (0.5)	1.6 (1.0)	1.4 (0.5)	<1.4 (-)
Free thyroxine (pmol/l)	13.4 (6.3)	14.7 (7.6)	17.2 (5.6)	19.9 †† (6.6)
Thyrotrophin (ng/ml)	5.2 (1.5)	5.3 (2.3)	4.9 (2.2)	5.3 (1.6)

* Calculated from thyroxine/thyroxine binding capacity.
† Statistically significantly different from control ($p<0.05$; t test).
†† Statistically significantly different from control ($p<0.01$; t test).

Table 5

Effects of Clofentezine on Thyroid Function in Male Rats Treated with Clofentezine in the Diet for 6 Weeks

	Dose level (ppm) 0	400	30,000
Tri-iodothyroxine (nmol/l)	0.9	1.0	1.1*
Thyroxine (nmol/l)	54	62*	69***
Thyroxine binding capacity	0.77	0.79	0.71
Free thyroxine index†	71	84	98**
Thyrotrophin (ng/ml)	5.4	5.7	9.0*

* Statistically significantly different from control ($p<0.05$; t test).
** Statistically significantly different from control ($p<0.01$; t test).
*** Statistically significantly different from control ($p<0.001$; t test).
† Calculated from thyroxine/thyroxine binding capacity.

C. Thyroid Hormone Clearance

A metabolism study in which rats were given daily doses of 20 mg clofentezine per kg body weight for up to 10 days showed that the pesticide and its metabolites did not accumulate in the thyroid gland. In another study, thyroid iodine uptake was increased by clofentezine after 4 months in rats given a 30,000 ppm diet (see Table 6). These results suggested that the pesticide did not directly affect the thyroid gland.

Table 6

Iodine Uptake in the Thyroid Gland (Mean cpm/Thyroid) in Clofentezine-Treated Male Rats.

Observation	Dose (ppm)* 0	30,000
6 hours after ^{125}I-thyroxine administration	113,400 ±23,600	189,600 ±54,600 †
24 hours after ^{125}I-thyroxine administration	163,500 ±53,700	210,200 ±65,000

† $P < 0.01$, Mann Whitney test.

Additional experiments were conducted to characterize the indirect effects of clofentezine on the thyroid. Some of those studies in which male rats were given diets containing 0 or 30,000 ppm clofentezine for two to five weeks followed by an intravenous dose of ^{125}I-thyroxine showed the following:

1. Bile flow rate in clofentezine treated rats was nearly twice that in control rats.

2. Fecal excretion of ^{125}I label over a 72 hour period rose from approximately 26% of the administered radioactivity in control rats to 40% in pretreated rats. Urinary excretion fell from 27% in the control rats to 15% of the administered thyroxine dose in pretreated rats. Total recovery of the radiolabel in urine and feces of control rats is approximately 53% in the control group and 55% in the clofentezine treated group.

3. The mean concentration of radioactivity in the blood of test rats 72 hours after administration was reported to be 0.058 and 0.029 ug/kg in the control and pretreated groups, respectively.

Thyroxine half-life values (time required for half of a given concentration of (^{125}I)-thyroxine to be eliminated from the body) were measured in two groups of male rats before and after they were given diets containing 0 or 30,000 ppm clofentezine for a period of 29 days. Table 7 summarizes the results. No statistically significant differences between the treated and control group were noted after clofentezine administration, but there was a statistically significant difference with respect to the mean change in measurements before and after clofentezine treatment. This statistically

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significant change can not be considered biologically significant without statistically significant differences in the half-life values determined after the 29-day treatment period.

Table 7

Mean Half-life (hours) for Thyroxine in the Blood of Clofentezine-Treated Male Rats.

	Dose (ppm)*	
	0	30,000
Before treatment with clofentezine	16.70 ±1.09	17.05 ±1.08
After treatment with clofentezine	17.61 ±1.62	16.42 ±1.44
Mean change (hours) during 1 month test period	+ 0.91 ±1.91	- 0.64 ** ±1.18

* Test diets were administered to rats for one month.
** $P < 0.05$, Mann Whitney test.

A greater proportion of administered ^{125}I recovered in the bile of pre-treated rats was associated with thyroxine metabolites than was observed in untreated control rats (see Table 8). This conclusion was based on the decreased amount of thyroxine associated ^{125}I identified in the bile. The amounts of glucuronide conjugate recovered from control and clofentezine treated rats were similar which is not consistent with the hypothesis that thyroxine metabolism was increased by clofentezine treatment, and other metabolites were not identified to support the conclusion.

Table 8

Proportion of ^{125}I Associated with Thyroxine and Its Metabolites in Bile from Clofentezine-Treated Male Rats.

Observation	Dose (ppm)	
	0	30,000
Thyroxine	16.52	5.55
Thyroxine glucuronide	11.73	11.91
Total identified	28.25	17.46
Per cent of administered radiolabel recovered from bile **	6.4	10.2

* Per cent of total amount of radiolabeled iodine in the bile recovered during a 4-hour period following administration of ^{125}I-thyroxine.
** Total amount of dose recovered during a 4-hour period.

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There was an increase in the iodine uptake by thyroid glands in male rats treated with clofentezine (see Table 9) indicating that the chemical is associated with an increase in synthesis of thyroid hormones.

Table 9

Thyroid Iodine Uptake (mean cpm/thyroid)

	Dose (ppm)*	
	0	30,000
6 hours after ^{125}I-thyroxine administration	113,400 ±23,600	189,600 ±54,600 †
24 hours after ^{125}I-thyroxine administration	163,500 ±53,700	210,200 ±65,000

† $P < 0.01$, Mann Whitney test.

These studies indicate that clofentezine increased the bile flow rate, shifted the excretion pattern toward the feces, and reduced blood levels of exogenous thyroxine. However, thyroxine half-life values were not significantly decreased, and the total excretion of administered thyroxine for control and clofentezine treated rats was not greatly altered by a dietary level of 30,000 ppm.

Although these results suggest that clofentezine changes the excretion pattern and possibly the metabolism for exogenous thyroxine, they do not clearly demonstrate that the pesticide enhances clearance of thyroxine in rats.

D. Progression of Lesions

As noted above in Section III. A., the incidence and severity of hypertrophy in the chronic feeding study was dose-related (see Table 2). Although there were no statistically significant differences or trends, the incidence of hyperplasia suggested a dose-related trend for that lesion could be possible over a broader dose range than the one used in the chronic feeding study.

E. Other Studies

1. Reversibility of Thyroid Lesions

There were limited data suggesting that hypertrophy is reversible in male rats given diets containing 3000, 9000, or 27,000 ppm clofentezine for 9 weeks (see Table 10). Similar results were obtained with observations of colloid depletion (see Table 11).

Table 10

Reversible of follicular cell hypertrophy in rats given diets containing clofentezine for various times.

Observation	Dose level (ppm) 0	3000	9000	27,00
After 9 weeks				
Number examined	5	5	4	3
Absent	2	0	0	0
Minimal	1	0	0	0
Slight	1	3	1	1
Moderate	1	2	3	2
Severe	0	6	0	0
Very Severe	0	0	0	0
After 13 weeks				
Number examined	8	10	10	10
Absent	1	0	0	0
Minimal	2	0	0	0
Slight	3	4	2	5
Moderate	2	6	8	5
Severe	0	0	0	0
Very Severe	0	0	0	0
After 9 weeks + 4 weeks recovery				
Number examined	5	4	4	4
Absent	1	2	2	1
Minimal	0	0	0	1
Slight	3	2	2	2
Moderate	1	0	0	0
Severe	0	0	0	0
Very Severe	0	0	0	0

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Table 11

Reversible of colloid depletion in thyroid glands of male rats given diets containing clofentezine for various times.

Observation	Dose level (ppm) 0	3000	9000	27,00
After 9 weeks				
Number examined	5	5	4	3
Absent	4	0	0	0
Minimal	1	0	0	0
Slight	0	1	1	1
Moderate	0	2	0	1
Severe	0	2	3	1
Very Severe	0	0	0	0
After 13 weeks				
Number examined	8	10	10	10
Absent	4	0	0	1
Minimal	2	1	0	2
Slight	2	0	3	1
Moderate	0	3	2	6
Severe	0	6	5	0
Very Severe	0	0	0	0
After 9 weeks + 4 weeks recovery				
Number examined	5	4	4	4
Absent	1	2	2	0
Minimal	0	1	0	2
Slight	1	1	2	1
Moderate	3	0	0	0
Severe	0	0	0	1
Very Severe	0	0	0	0

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2. Liver Activity

Section I. A. above stated that clofentezine is expected to increase liver enzyme activity causing increased metabolism of thyroxine. Liver microsomal and mixed function oxidase enzyme activity was measured in several studies in rats given clofentezine in their diets for 2 to 10 weeks at levels of 10 to 30,000 ppm.

Dietary levels of 400 and 30,000 ppm clofentezine administered for 4 weeks increased the activity of uridine diphosphoglucuronate glucuronyl transferase (UDPGT) as indicated by the decrease in absorbance of nitrophenol at 405 nm (change in absorbance/minute/miligram microsomal protein). Group mean values reported for the 0, 10, 40, 400, and 30,000 ppm dose levels were 24, 27, 26, 43, and 101, respectively. The means for the 400 and 30,000 ppm dose groups were statistically significantly different from the control group mean at $p<0.01$ and $p<0.001$, respectively.

In two other studies, male rats were given diets containing 0, 10, 40, or 400 ppm for 2 weeks. There were statistically significant increases in cytochromes P_{450} and B_5 concentrations and in ethoxycoumarin de-ethylase and aldrin epoxidase activities (see Table 12 below). Liver weights relative to body weight were also statistically significantly increased by the 400 ppm dose level.

After 8 weeks on diets containing 0, 40, or 27,000 ppm clofentezine, male rats showed increases in aniline dehydroxylase activity and cytochrome P_{450} and cytochrome B_5 concentrations. In the 27,000 ppm dose group, aniline dehydroxylase activity was approximately double that of the control group, and the cytochrome P_{450} concentration was increased by approximately 50% in comparison to the control group.

F. Structure Activity Relationships

Clofentezine is not structurally similar to other chemicals known to have goitrogenic activity.

III. Summary

Because of the increased incidence of thyroid tumors in male rats in the chronic feeding study, re-evaluations of thyroid slides and additional studies of clofentezine's effects on the thyroid were conducted.

The re-evaluations of slides from rats in chronic and subchronic studies indicated that goitrogenic effects of the pesticide included dose-related increases in the incidence and severity of follicular cell hypertrophy, and an increased incidence of hyperplasia in treated male rats. In short-term studies at high doses (3,000 to 30,000 ppm), follicular cell hypertrophy was reversed in the three weeks following a 9-week treatment period. Observation of hyperplasia was limited to the chronic feeding study, and its incidence was not dose-related. Thyroid weights were increased along with these microscopic changes but not in a statistically significant manner.

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Table 12

Mixed Function Oxidase Activity in Male Rats after Two Weeks of Dietary Treatment with Clofentezine

Observation	Dose level (ppm) 0	10	40	400
First study				
Liver weight (g/100 g body weight)	2.82	2.96	3.02	3.31**
Microsomal protein concentration (mg/g liver)	34.5	28.6**	31.7	32.6
Aniline dehydroxylase activity (n moles/min/mg protein)	0.26	0.22	0.22	0.28
p-Nitroanisole demethylase activity (n moles/min/mg protein)	6.05	6.51	5.73	6.52
Cytochrome P_{450} (n moles/g liver)	0.64	0.65	0.78**	0.73*
Cytochrome B (n moles/mg protein)	0.21	0.27*	0.31*	0.33**
Second study - first trial				
Liver weight (g/100 g body weight)	2.49	2.53	2.60	2.74**
Protein concentration (mg/g liver)	25.4	26.1	22.1*	28.4*
Ethoxycoumarin de-ethylase (nmoles/min/mg protein)	2.23	2.68	2.72*	3.34**
Aldrin epoxidase (nmoles/min/mg protein)	2.52	2.39	2.38	3.33*
Cytochrome P_{450} (n moles/g liver)	0.56	0.59	0.59	0.76**
Cytochrome B (n moles/mg protein)	0.24	0.26	0.27	0.36**
Second study - second trial				
Liver weight (g/100 g body weight)	2.61	2.56	2.56	2.62
Protein concentration (mg/g liver)	18.9	20.1	19.3	21.3**
Ethoxycoumarin de-ethylase (nmoles/min/mg protein)	1.31	1.50	1.40	1.57*
Aldrin epoxidase (nmoles/min/mg protein)	0.42	0.46	0.45	0.52*
Cytochrome P_{450} (n moles/g liver)	0.54	0.58	0.60	0.67**
Cytochrome B (n moles/mg protein)	0.24	0.20*	0.19*	0.27

*Significantly different from controls ($p<0.05$).
**Significantly different from controls ($p<0.01$).

Clinical chemistry at the end of the chronic feeding study showed a statistically significant increase in free thyroxine in amle rats given the 400 ppm dose level without an increase in TSH levels. A high dose of clofentezine (30,000 ppm) administered for 6 weeks increased thyroxine, tri-iodothyroxine and TSH blood levels, but these increases are not consistent with a classical thyroid-pituitary hormone imbalance such as low thyroxine levels associated with elevated TSH levels or high thyroxine associated with low TSH levels.

Clofentezine did not accumulate in the thyroid glands of treated rats, and the rate of iodine uptake was increased by the chemical. These results suggested that the chemical indirectly affected the thyroid gland in rats, and several short-term studies were conducted to characterize that effect.

In some of theose studies, the pesticide increased the bile flow rate, shifted the excretion pattern of thyroxine and its metabolites toward the feces, and reduced blood levels of exogenous thyroxine. However, the rate of clearance for exogenous thyroxine was not significantly increased, and the total recovery of administered thyroxine for control and clofentezine treated rats was not greatly increased by a dietary level of 30,000 ppm (75 times the highest dose in the chronic feeding study). Although these results suggest that clofentezine changes the excretion pattern and possibly the metabolism for exogenous thyroxine, they do not demonstrate that the effects are biologically significant in terms of the conditions of the chronic feeding study (i. e., much lower dose levels and much longer treatment of test animals).

Since the first step in the proposed mechanism for thyroid tumors associated with clofentezine is liver enzyme induction, several liver function studies were considered. These experiments indicated that dose levels from 400 ppm to 30,000 ppm increased the concentration of cytochromes P_{450} and b_5 and increased the activity of anilin dehydrogenase, uridine diphosphoglucuronate glucuronyl transferase (UDPGT), ethoxycoumarin de-ethylase and aldrin epoxidase. These results are consistent with increased bile flow and changes in the thyroxine excretion pattern.