Triprolidine: 104-Week Feeding Study in Rats

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The antihistamine, triprolidine hydrochloride, was fed at dietary concentrations of 0, 250, 1000, or 2000 ppm (as the free base) to groups of 60 Fischer 344 (F344) rats of each sex for up to 2 years to evaluate its potential carcinogenicity. Up to 12 ppm per sex from each group were killed at 65 weeks, and hematology, clinical chemistry, and histopathology were evaluated. A complete histopathological evaluation was performed on all other animals; survivors were kept under observation for 2 years. Survival was significantly extended in triprolidine-treated males and females, particularly at the high dose. At the close of the study high-dose males and females had gained significantly less body weight than controls. Among rats killed at 65 weeks females in the mid- and high-dose groups weighed significantly less than controls, but weights of control and exposed males were not significantly different. The incidences of numerous lesions tended to decrease with increasing triprolidine dose. In females, clitoral gland adenomas, thyroid c-cell hyperplasia and neoplasia, mammary gland hyperplasia and fibroadenomas, and uterine stromal polyps, and in males, anterior pituitary gland adenomas, preputial gland neoplasia, thyroid c-cell hyperplasia, pancreatic islet neoplasia, mononuclear cell leukemia, and the combination of lymphocytic, histiocytic, and undifferentiated cell malignant lymphomas and mononuclear leukemia, all exhibited negative dose trends. Cytoplasmic alterations of the parotid gland and numerous liver lesions tended to be more frequent in treated than control animals. Liver lesions that exhibited positive dose trends include chronic inflammation and centrilobular fatty change in both sexes, mixed cell foci, and the combination of mixed cell foci and eosinophilic foci in females, and in males, basophilic foci and eosinophilic foci. Triprolidine was not carcinogenic in F344 rats.

Triprolidine hydrochloride (CAS No. 6138-79-0) is an alkylamine with antihistaminic activity. Antihistamines antagonize smooth muscle-stimulating actions of histamine in the gastrointestinal tract, bronchial muscle, and blood vessels and block histamine-induced increases in capillary permeability (Gilman et al., 1985). They are used in allergic disorders to relieve symptoms of histamine release. The hydrochloride form of triprolidine is used in drugs, and annual usage in the United States is estimated to be over 2 million tablets. Usual oral doses, given 3 or 4 times daily, are 2.5 mg for adults, 1.25 mg for children 6 to 12 years old, and 0.3 to 0.9 mg for younger children.

No appreciable mutagenic activity, either in the absence or presence of S9, has been demonstrated for either triprolidine or a major metabolite, hydroxymethyltriprolidine, in Salmonella typhimurium tester strains TA97, TA98, TA100, or TA104 (Hansen et al., 1988).

Triprolidine hydrochloride has widespread use in over-the-counter drug formulations and belongs to the class of antihistamines that is most often used. It was nominated for testing under the auspices of the National Toxicology Program because toxicological testing of this compound has been very limited and it is functionally related to methapyrilene, another antihistamine that has been shown to be a liver carcino gen in Fischer 344 rats (Lijinsky et al., 1980).

MATERIALS AND METHODS
Chemical and Diets

Triprolidine hydrochloride monohydrate, purchased in two lots from Chemical Dynamics Corporation (South Plainfield, NJ, Lot No. 120223) and Balenco Enterprises, Inc. (Compton, CA, Lot No. 004-110), was >99% pure, as determined using high-pressure liquid chromatography (HPLC) and gas chromatography/flame ionization detection (GC/FID); chemical structure was confirmed using mass spectral analysis. Analyses at the time of receipt and after termination of use of the different lots of the pure compound revealed no decomposition during storage.

The compound was dissolved in 95% ethanol (USP) and mixed into autoclaved NIH-31 feed with a “V” blender at a ratio of 1 ml of solution to 10 g of feed. Control feed was mixed with an equal proportion of ethanol. Ethanol was removed under vacuum during the mixing process. Homogeneity was determined on a single diet batch, mixed to contain 250 ppm triprolidine. Its average concentration was 242 ppm (n = 10); the coefficient of variation was 1.1%. No significant loss in the concentration of triprolidine in formulated feed was found after 16 weeks of storage, and maximum storage during the study was 8 weeks, at 70°F. Since each batch

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of feed was analyzed for triprolidine (Thompson and Holder, 1984) prior to its use, no feed was used if outside ±10% of the target concentration. Average concentrations were no more than 4% removed from the target concentrations, and coefficients of variation ranged from 3.2 to 3.4%.

**Study Design**

Feed was selected as the vehicle of exposure since human administration is by multiple daily oral doses. Diets containing 0, 250, 1000, or 2000 ppm triprolidine (free base) were fed to groups of 12 male and 12 female rats for up to 65 weeks, and to groups of 48 male and 48 female rats for 103 weeks followed by 1 week of control diet. Rats were fed dosed diet until the day before they were euthanized at 65 weeks. Animals were fasted overnight before euthanizing at either 65 or 104 weeks.

**Animals and Animal Maintenance**

Male and female F344 rats were produced at the National Center for Toxicological Research (NCTR). Rats were randomly assigned, three per cage, and dosing was started when they were 5 to 6 weeks old. Males and females were housed on separate racks and maintained in the same room with mice receiving triprolidine. Cages were rotated within each column by moving each cage one shelf level every other week. Feed and water were available on demand. Feeders and feed were replaced weekly; fresh water, water bottles, cages, and bedding were replaced twice weekly. The feeder used excluded the accumulation of bedding on the surface of the feed. Feed consumption was measured for each cage weekly by weighing the feeder immediately after adding fresh feed and just prior to replacement of that feeder at the close of the week. Spillage was estimated semiquantitatively on five cages from each dose group once a week during the first 13 weeks of the study and during the final 4 weeks of the study. Estimates were made by visual comparison of each animal cage with a set of standard cages, each containing the standard amount of bedding and a measured quantity of feed. The animal room was controlled within ranges of 70–74°F and 40–60% relative humidity (averages, 72°F and 49%, respectively).

**Microbiological Surveillance**

A microbiological/parasitic survey of animals was accomplished by using 12 male and 12 female sentinel rats. These rats received control feed, and one per month was removed for evaluation (sex was alternated monthly). No bacterial pathogens or parasites were detected. However, 5 rat serum specimens were serologically identified as positive for *Mycoplasma arthritidis* and one was found positive for rat coronavirus, but no overt symptoms of disease were reported. Microbiological surveillance of the experiment also was achieved through periodic evaluation of room air, surface swabs, animal cage waste, and cage water. Room swabs never revealed pathogens, and only 1 of 53 swabs exceeded bacterial contamination of 100 colony-forming units (CFU). Room air samples never exceeded standards of 100 bacteria or 20 mold CFU per 15 cubic feet of air. *Pseudomonas aeruginosa* was recovered from 1 of 52 composited cage waste samples representing 250 individual rat cages. *P. aeruginosa* was also recovered from 61 of 1561 cage water samples; but, no evidence of symptoms associated with this organism were ever noted.

**Clinical Examinations and Pathology**

Cages were observed twice daily to detect dead or moribund rats. Rats were weighed weekly during the first 3 months and final 2 weeks of the experiment and, otherwise, were weighed once or twice monthly. Animals were euthanized by CO₂ overexposure, and necropsies were performed.

Blood was collected for hematologic evaluation and serum enzyme analy-

sis from rats killed at 65 weeks. Alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, amylase, and lactate dehydrogenase were analyzed. In addition, brain, liver, kidneys, thymus, and spleen were weighed after fixation in formalin; both kidneys were weighed together. Blood was collected from rats killed at 104 weeks for hematology; no clinical chemistry was done and no organs were weighed.

All organs and tissues were examined for gross lesions during necropsy. Tissues were preserved in 10% neutral-buffered formalin, trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A complete histological evaluation of over 40 tissues was performed on dead and moribund rats, control and high-dose animals killed at 65 weeks, and on all dose groups killed at 104 weeks. All gross lesions were examined histologically.

**Statistical Methods**

**Mortality analyses.** The probability of mortality was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals removed for reasons other than death or morbidity were censored from the mortality analyses at the time of their removal. Analyses for dose-related effect on mortality used the method of Cox (1972) to test two groups for equality and Tarone's (1975) test for a dose-related trend.
the following analyses were performed: (1) A Cochran-Armitage trend test (Armitage, 1971; Snedecor and Cochran, 1980) to determine if there was a positive (increasing incidence or prevalence with increasing dose) trend across dose groups. This was a one-sided test for positive trend, and negative trend was tested by using the complement of the observed p value. (2) Fisher’s exact test (Everitt, 1977) was calculated for comparing the control group to each of the dose groups. (3) For the overall group only, a time-to-appearance analysis for the prevalence of the lesion was done using PROC CHRONIC (Kodell et al., 1983). This also is a one-sided test for positive trend, but the symmetry of the test allows testing for negative trend as above. The analysis was done for an overall trend across doses and comparing each dose to control. For lesions that were graded as a cause of death, tests for both mortality and time-to-appearance of the lesions were done.

RESULTS

Body Weights, Water and Feed Consumption

Body weight curves of animals scheduled to be killed after 104 weeks on experiment are presented in Fig. 1. Weight gain was inhibited in males and females fed 2000 ppm triprolidine. Statistical comparisons of weight gain between the first and last weeks of the study revealed significant (at the 0.01 level) inhibition (>20%) in both males and females fed 2000 ppm when compared to controls. Similar comparisons made over the first 13 weeks of the study showed significant weight gain inhibition (>15%) only in males fed 2000 ppm triprolidine.

Weekly feed consumption, estimated only by the reduction in feeder weight, was not affected by triprolidine when expressed relative to body weight. Average weekly consumption (g/g body weight) ranged from 0.37 to 0.40 in females and from 0.32 to 0.34 in males. However, separate semiquantitative measurements of feed spillage made during the first 3 months and last several weeks of the experiment (Table 1) suggested that spillage was dose related, highly

### TABLE 1
Feed Spillage by F344 Rats Fed Triprolidine

| Time period | Treatment | Spillage (%) |
|-------------|-----------|--------------|
|             |           | Females      | Males       |
|             |           | Mean* | Range* | Mean* | Range* |
| 1–13 weeks  | Control   | 12.0  | 7–27   | 9.2   | 5–24   |
|             | 250 ppm   | 12.5  | 7–29   | 8.5   | 5–26   |
|             | 1000 ppm  | 13.0  | 7–41   | 8.9   | 4–25   |
|             | 2000 ppm  | 18.6  | 7–44   | 13.3  | 5–32   |
| 101–104 weeks | Control | 35.1  | 31–51  | 39.0  | 18–62  |
|             | 250 ppm   | 30.6  | 13–44  | 38.2  | 28–67  |
|             | 1000 ppm  | 38.3  | 20–76  | 34.6  | 24–49  |
|             | 2000 ppm  | 33.6  | 16–49  | 43.1  | 24–60  |

*Mean of five cages during the specified period.
*Range of individual weekly cage observations.
variable, and quite different at different times in the experiment. Weekly spillage averages by animals fed the highest dose of triprolidine were greater than those of the other dose groups during the first three months of the study. Subsequent measurements, at the termination of the study, showed no clear dose dependency; however, spillage at the end of the experiment was clearly higher than that during the first 8 weeks of the experiment. Due to high variability of spillage measurements, the variation with time, and failure to measure spillage throughout the whole experiment, daily triprolidine dosages were estimated based upon the reduction of feeder weight, not adjusted for spillage. In females the estimated daily triprolidine dosages were 13 (250 ppm), 52 (1000 ppm), and 115 (2000 ppm) mg/kg. In males the dosages were 11 (250 ppm), 46 (1000 ppm), and 96 (2000 ppm) mg/kg. Since spillage did take place, these clearly are overestimates of dosage.

However, the percentage overestimation is virtually the same for the two lower doses, and at 2000 ppm the overestimate would be less than 8% greater than for the lower doses.

**Survival and Clinical Signs**

Mortality curves are shown in Fig. 2. There were significant dose–response trends toward extension of the life span of both female (p < 0.05) and male (p < 0.001) treated animals. This tendency for triprolidine to extend the life span was especially apparent among animals fed the highest dose level. Pairwise statistical comparisons revealed that animals fed the 2000 ppm diet survived a significantly longer time than controls (p < 0.05 in both sexes).

**Organ Weights, Hematology, and Clinical Chemistry**

Significant organ weight findings are reported in Table 2. Body weights of females killed at 65 weeks were significantly lower in high- and mid-dose groups than those of controls, but no dose-related differences in absolute organ weights were noted. Although there were significant dose-related increases in organ to body weight ratios for brain, liver, kidney, and thymus, there were no significant changes in organ to brain weight ratios of females.

Body weights of males killed at 65 weeks were not significantly altered by triprolidine treatment, but both liver and spleen weights were influenced by triprolidine (Table 2). Liver, whether expressed as absolute weight or liver to body or brain weight ratio, was greater in males fed 2000 ppm triprolidine than in controls. Triprolidine treatment also was associated with a decrease in spleen weight, and spleen to body or brain weight ratio in males.

Since the hematology profile of nonleukemic rats at 104 weeks clearly differed from leukemic rats regardless of sex, leukemic animals were excluded from the analysis of hematology results. There was no effect of triprolidine on female hematology, either at 65 or 104 weeks, nor in males at 104 weeks. At 65 weeks males exhibited only a triprolidine-associated decrease in mean corpuscular volume (control, 57.3 ± 0.2; 250 ppm, 58.0 ± 0.4; 1000 ppm, 55.9 ± 0.3; 2000 ppm, 54.9 ± 0.2 μm³).

Compound-related differences in clinical chemistry observed in rats killed at 65 weeks are reported in Table 3. Significant pairwise differences in female serum lactate dehydrogenase (LDH) activity were found between low- and high-dose females, but there were no other differences. A significant dose effect was also noted for LDH-1 in females. Males showed a dose-related increase in sorbitol dehydrogenase indicative of altered liver function.

**Pathology**

Negative trends in females. Trend analyses revealed significant negative dose–response trends for numerous
TRIPROLIDINE BIOASSAY IN RATS

TABLE 2
Influence of Triprolidine on Body and Organ Weights of F344 Rats

| Parameter | 0       | 250     | 1000    | 2000    | Trend (p<) |
|-----------|---------|---------|---------|---------|------------|
| **Females** |         |         |         |         |            |
| Body      | 294.1 ± 17.2 | 302.8 ± 18.2 | 271.5 ± 22.8* | 242.2 ± 17.0* | 0.01       |
| Liver     | 12.8 ± 0.7  | 12.5 ± 1.2  | 13.3 ± 1.1  | 14.5 ± 1.5* |            |
| Spleen    | 0.97 ± 0.11 | 0.91 ± 0.10 | 0.88 ± 0.04 | 0.82 ± 0.04* | 0.01       |
| **Males** |         |         |         |         |            |
| Body      | 451.2 ± 17.1 | 436.5 ± 31.6 | 453.5 ± 19.0 | 444.2 ± 22.7 | ns         |
| Liver     | 12.8 ± 0.7  | 12.5 ± 1.2  | 13.3 ± 1.1  | 14.5 ± 1.5* |            |
| Spleen    | 0.97 ± 0.11 | 0.91 ± 0.10 | 0.88 ± 0.04 | 0.82 ± 0.04* | 0.01       |

Organ to body weight ratio

| Parameter | 0       | 250     | 1000    | 2000    | Trend (p<) |
|-----------|---------|---------|---------|---------|------------|
| **Females** |         |         |         |         |            |
| Brain     | 7.2 ± 0.4   | 7.2 ± 0.4   | 7.8 ± 0.6   | 8.9 ± 0.6*  | 0.01       |
| Liver     | 25.7 ± 1.1  | 25.6 ± 1.3  | 26.7 ± 2.4  | 28.3 ± 2.2* | 0.01       |
| Kidney    | 7.0 ± 0.4   | 6.9 ± 0.5   | 7.3 ± 0.4   | 7.9 ± 0.6*  | 0.01       |
| Thymus    | 0.32 ± .04  | 0.32 ± .05  | 0.34 ± .06  | 0.39 ± .07  | 0.05       |
| **Males** |         |         |         |         |            |
| Liver     | 28.4 ± 1.1  | 28.6 ± 2.8  | 29.3 ± 2.0  | 32.6 ± 2.3* | 0.01       |
| Spleen    | 2.2 ± 0.2   | 2.1 ± 0.3   | 1.9 ± 0.1   | 1.8 ± 0.1*  | 0.01       |

Organ to brain weight ratio

| Parameter | 0       | 250     | 1000    | 2000    | Trend (p<) |
|-----------|---------|---------|---------|---------|------------|
| **Males** |         |         |         |         |            |
| Liver     | 5.4 ± 0.3  | 5.4 ± 0.6  | 5.7 ± 0.5  | 6.2 ± 0.7* | 0.01       |
| Spleen    | 0.41 ± 0.05 | 0.39 ± 0.04 | 0.37 ± 0.02 | 0.35 ± 0.02* | 0.01       |

* Significantly differs from control, p < 0.01.
* Significantly differs from control, p < 0.05.

Histopathologic lesions among females killed at 104 weeks. Triprolidine treatment was associated with trends toward decreased prevalence of thyroid c-cell hyperplasia, thyroid c-cell neoplasia (adenomas or carcinomas) (Table 4), mammary gland hyperplasia, mammary fibroadenomas, uterine stromal polyps, and the combination of leukemia and malignant histiocytic lymphoma (Table 5). Pairwise comparisons revealed, in several instances, that these negative trends were supported by prevalences in the high-dose group that were significantly lower than the control prevalence. This was true for thyroid c-cell hyperplasia and neoplasia (Table 4) and for mammary gland hyperplasia (Table 5).

Time adjusted prevalences of the overall groups (including dead, moribund, and euthanized animals from each dose group), similarly revealed negative trends for thyroid c-cell hyperplasia (p < 0.001) and neoplasia (p < 0.05) (Table 4), mammary gland hyperplasia (p < 0.01) and neoplasia (p < 0.05), clitoral gland adenomas (p < 0.05), and uterine stromal polyps (p < 0.05) (Table 5). In each of these instances, except clitoral gland adenomas, the time adjusted prevalence in the high treatment group was significantly lower (p < 0.05 to <0.001) than in controls. In addition, there was a significant (p < 0.01) trend toward a dose-related extension of the time of onset of mammary fibroadenomas, and time

TABLE 3
Clinical Chemistry Changes Associated with Triprolidine Treatment of F344 Rats for 65 Weeks

| Concentration (ppm) | Females | Males: |        |        |
|---------------------|---------|--------|--------|--------|
|                     | LDH (IU/liter) | LDH-1 (IU/liter) | SDH (IU/liter) |        |
| 0                   | 81.5 ± 9.1 | 10.8 ± 0.9 | 13.6 ± 2.0 |        |
| 250                 | 32.0 ± 8.2 | 9.8 ± 0.5 | 13.2 ± 1.6 |        |
| 1000                | 48.8 ± 12.5 | 10.5 ± 0.5 | 18.8 ± 2.0 |        |
| 2000                | 94.9 ± 16.3 | 8.4 ± 0.6 | 27.6 ± 2.7 |        |

Note. Abbreviations used: LDH, lactate dehydrogenase; SDH, sorbitol dehydrogenase.
of onset was significantly \( p < 0.05 \) longer in high-dose than in control animals.

**Negative trends in males.** Among male rats killed at 104 weeks, negative dose–response trends were found in the prevalences of anterior pituitary gland adenomas, thyroid \( \alpha \)-cell hyperplasia, and preputial gland neoplasms (Table 6). Furthermore, there were significantly fewer pituitary adenomas and preputial neoplasms in the high-dose group compared to controls, when pairwise comparisons were made. When dead and moribund animals were combined with euthanized animals for analysis, time-adjusted prevalences for these same lesions showed similar negative trends \( p < 0.01 \) to \( p < 0.05 \), and pairwise comparisons also revealed significantly reduced prevalences in high-dose animals compared to controls. There was also a highly significant \( p < 0.001 \) delay in the time of onset of anterior pituitary adenomas that was particularly apparent in the high-dose males (Table 6).

In addition, when the overall dose groups were analyzed, pancreatic islet carcinomas, mononuclear cell leukemia, and the combination of mononuclear cell leukemia or lymphocytic, histiocytic, or undifferentiated cell malignant lymphomas (Table 6) gave evidence of either reduced prevalence or delayed appearance. For leukemia and the combination of leukemia and lymphoma, there were significant \( p < 0.01 \) and \( p < 0.001 \), respectively) trends toward delay in onset of and death due to the lesions, and onset and death were significantly delayed \( p < 0.05 \) and \( p < 0.01 \), respectively) in the high-dose group compared to controls for both of these comparison groups. Thus, early mortality due to leukemia or the combination of leukemia and lymphomas appeared to be reduced by triprolidine treatment.

**Positive trends.** An increasing frequency of cytoplasmic alterations of the parotid gland was associated with increasing doses of triprolidine in both rat sexes (Tables 7 and 8). This histologic change was diffuse and characterized by enlargement of individual acinar cells resulting from increased cytoplasmic content. The cytoplasm of the most severely affected cases was finely granular and had a distinct blue–gray color. The nucleus of many of these cells was peripherally located. Although the incidence of these observations was dose-dependent, the severity was similar in all treated groups. It was noted primarily in rats surviving to the 104-week termination, where it was significantly more prevalent in both sexes fed the mid- and high-dose levels of triprolidine than in controls. The tendency for the finding to be less frequent among dead or moribund than among euthanized animals was noted even among rats removed shortly before the final euthanization and may be because terminally ill animals ate little or no food and were not

### TABLE 4

Histopathologic Observations Showing Negative Dose–Response Trends in Female F344 Rats Exposed to Dietary Triprolidine: Thyroid Glands

| Lesion                        | Control | 250 ppm | 1000 ppm | 2000 ppm |
|-------------------------------|---------|---------|----------|----------|
| Thyroid \( \alpha \)-cell hyperplasia\(^{a}\) |         |         |          |          |
| Terminal                      | 20/28\(^c\) | 23/29   | 20/32    | 12/36\(^c\) |
| Overall                       | 37/57   | 31/44   | 28/46    | 15/57    |
| Thyroid \( \alpha \)-cell carcinoma or adenoma\(^{a}\) |         |         |          |          |
| Terminal                      | 11/28\(^c\) | 8/29    | 6/32     | 1/36\(^c\) |
| Overall                       | 13/57   | 10/44   | 8/46     | 4/57     |

\(^{a}\) Denominators represent only animals whose tissue was examined histologically.

\(^{b}\) Animals from 65-week euthanized group were excluded from overall summary because tissue was examined only if grossly visible lesions were present or animal was dead or moribund.

\(^{c}\) Hyperplasia was recorded only for rats without bilateral thyroid neoplasia.

\(^{d}\) Significant trend \( p < 0.001 \).

\(^{e}\) Significantly differs from control \( p < 0.01 \).

\(^{f}\) Numerator is number of animals with one or more of these lesions.

\(^{g}\) Significantly differs from control \( p < 0.001 \).

\(^{h}\) One carcinoma was present. All other neoplasms were adenomas.

### TABLE 5

Histopathologic Observations Showing Negative Dose–Response Trends in Female F344 Rats Exposed to Dietary Triprolidine

| Lesion                              | Control | 250 ppm | 1000 ppm | 2000 ppm |
|-------------------------------------|---------|---------|----------|----------|
| Mammary gland, fibroadenoma\(^{a}\) |         |         |          |          |
| Terminal                            | 9/28\(^e\) | 12/29   | 12/32    | 5/37     |
| Overall                             | 15/60   | 19/59   | 15/60    | 5/59     |
| Mammary gland, hyperplasia          |         |         |          |          |
| Terminal                            | 21/26\(^e\) | 17/28   | 20/30    | 18/35\(^g\) |
| Overall                             | 39/58   | 29/45   | 28/45    | 25/57    |
| Clitoral gland, adenoma or bilateral adenoma\(^{a}\) |         |         |          |          |
| Overall                             | 3/58    | 7/44    | 3/45     | 0/58     |
| Uterus, stromal polyp                |         |         |          |          |
| Terminal                            | 5/28\(^e\) | 7/29    | 7/32     | 2/37     |
| Overall                             | 8/60    | 7/47    | 7/47     | 2/59     |
| Mononuclear cell leukemia or malignant histiocytic lymphoma\(^{a}\) |         |         |          |          |
| Terminal                            | 6/28\(^e\) | 7/29    | 4/32     | 3/37     |

\(^{a}\) With the exception of mammary gland fibroadenomas, animals from 65-week euthanized group were excluded from overall summary because tissue was examined only if grossly visible lesions were present or the animal was dead or moribund.

\(^{b}\) Denominator is the number of rats necropsied.

\(^{c}\) Numerator is number of rats with one or more of these lesions.

\(^{d}\) Significant trend \( p < 0.05 \).

\(^{e}\) Denominator is number of rats whose tissue was examined histologically.

\(^{f}\) Significantly different from controls \( p < 0.05 \).
TABLE 6
Histopathologic Observations Showing Negative Dose–Response Trends in Male F344 Rats Exposed to Dietary Triprolidine

| Lesion*                | Control 250 ppm | 1000 ppm | 2000 ppm |
|------------------------|-----------------|----------|----------|
| Anterior pituitary gland, adenoma | 14/209 | 11/17 | 12/26 | 11/35 |
| Overall                | 31/55 | 23/45 | 20/46 | 16/56 |
| Thyroid c-cell hyperplasia | 14/209 | 12/17 | 15/26 | 16/35 |
| Overall                | 27/52 | 23/42 | 18/43 | 21/57 |
| Preputial gland, adenoma | 3/19 | 0/17 | 0/25 | 0/35 |
| Overall                | 5/58 | 1/48 | 2/46 | 0/58 |
| Preputial gland, adenoma or adenocarcinoma* | 3/19 | 0/17 | 0/25 | 0/35 |
| Overall                | 5/58 | 1/48 | 2/46 | 0/58 |

* Denominator is number of rats with histologically examined tissue except for combination of leukemia and lymphomas. In this instance denominator is number of rats necropsied.

* With the exception of leukemia and leukemia/lymphoma categories, animals from 65-week euthanized group were excluded from overall summary since tissue was examined only if grossly visible lesions were present or the animal was removed as dead or moribund.

* Significant trend (p < 0.01).

* Significantly different from controls (p < 0.01).

* Significant trend (p < 0.05).

* Significantly different from controls (p < 0.05).

* Significantly different from controls (p < 0.001).

* Numerator is number of animals with one or more of these lesions.

Numerous liver conditions were more frequent in triprolidine-treated than in control animals. The following liver observations showed positive dose–response trends in female F344 rats killed at 104 weeks: (1) chronic inflammation, (2) centrilobular fatty change, (3) basophilic foci, and (4) eosinophilic foci (Table 7). The positive trends for the latter two observations were not highly significant and were not supported by significance when individual comparisons were made between controls and specific dose groups. The following liver observations demonstrated positive dose–response trends in males: (1) chronic inflammation, (2) centrilobular fatty change, (3) basophilic foci, and (4) eosinophilic foci (Table 8).

TABLE 7
Histopathologic Observations Showing Positive Dose–Response Trends in Female F344 Rats Exposed to Dietary Triprolidine

| Lesion*                                      | Control 250 ppm | 1000 ppm | 2000 ppm |
|----------------------------------------------|-----------------|----------|----------|
| Cytoplasmic alterations of parotid gland      | 2/28*           | 5/29     | 10/32    | 28/37* |
| Overall                                      | 3/59 | 5/47 | 11/48 | 30/59 |
| Liver chronic inflammation                   | 16/28*          | 15/29    | 24/32    | 33/37* |
| Overall                                      | 32/60 | 18/47 | 30/48 | 44/59 |
| Liver centrilobular fatty change              | 0/28*           | 0/29     | 0/32     | 5/37 |
| Overall                                      | 0/60 | 0/47 | 0/48 | 6/59 |
| Liver mixed cell focus                       | 1/28*           | 3/29     | 3/32     | 8/37* |
| Overall                                      | 2/60 | 4/47 | 3/48 | 10/59 |
| Liver mixed cell focus or eosinophilic focus | 1/28*           | 3/29     | 4/32     | 8/37* |
| Overall                                      | 3/60 | 4/47 | 4/48 | 10/59 |

* Denominator is number of rats with histologically examined tissue.

* Animals from 65-week euthanized group were excluded from overall summary since tissue was examined only if grossly visible lesions were present or animal was dead or moribund.

* Significant trend (p < 0.001).

* Significantly different from controls (p < 0.05).

* Significantly different from controls (p < 0.01).

* Significantly different from controls (p < 0.001).

* Significant trend (p 0.01).

* Significant trend (p 0.05).

* Numerator is number of animals with one or more of these lesions.
Histopathologic Observations Showing Positive Dose–Response Trends in Male F344 Rats Exposed to Dietary Triprolidine

| Lesion*          | Control 250 ppm | 1000 ppm | 2000 ppm |
|------------------|-----------------|----------|----------|
| Cytoplasmic alterations of parotid gland | 4/20* | 8/17 | 13/26* | 26/35* |
| Terminal         | 5/56 | 9/45* | 14/46* | 30/58  |
| Overall          | 10/20* | 1/17' | 14/26 | 26/35  |
| Liver chronic inflammation | 24/59 | 13/60 | 29/59 | 42/59  |
| Terminal         | 0/20* | 0/17 | 1/26 | 8/35* |
| Overall          | 0/59 | 4/60 | 9/59 | 2/25  |
| Liver basophilic focus | 5/59 | 1/60 | 8/59 | 11/59  |
| Overall          | 4/20* | 1/17 | 3/26 | 11/35 |
| Liver eosinophilic focus | 5/59 | 1/60 | 3/59 | 12/59  |

* Denominator is number of rats with tissue histologically examined except for the mammary gland. Here the denominator is number of rats necropsied.

a Significant trend (p < 0.001).

b Significant different from controls (p < 0.05).

Denominator is number of rats with tissue histologically examined except for the mammary gland. Here the denominator is number of rats necropsied.

in both cases the prevalence and/or time-adjusted prevalence in high-dose animals significantly differed from controls. In addition, the time-adjusted prevalence of centrilobular fatty change in the mid-dose group significantly differed from controls.

No positive dose–response trends for neoplastic endpoints were detected in either sex.

**DISCUSSION AND CONCLUSIONS**

Subchronic studies carried out in F344 rats (Jackson et al., 1993) at dietary levels of 250, 500, 1000, 2000, and 4000 ppm triprolidine were very predictive of observations made in the current study. In the 90-day study body weight gain suppression of about 10% or more was found in females at 1000 ppm and above and in males at 2000 and 4000 ppm. Histologically two target organs were identified in both male and female rats. Mild to moderate fatty changes of the liver were noted in males and females fed 4000 ppm triprolidine and only in males fed the 2000 ppm level. These changes were suggestive of direct liver cell injury caused by triprolidine followed by or associated with fatty change. Cytoplasmic alterations were also observed in the parotid gland of male and female rats fed 2000 or 4000 ppm levels and in some males fed 1000 ppm triprolidine. These changes were considered more likely to be physiologic than pathologic. A low dose of 250 ppm was selected as a no effect level in both sexes for the current study and 2000 ppm was selected as the highest dose likely not to be life-threatening, especially in males.

In the current study the highest dose of triprolidine was clearly associated with reduced weight gain and extended life span in both male and female F344 rats. This extended life span was associated with lowered frequencies and/or reduced mortality from leukemia and/or lymphoma among treated males and females and especially in high-dose males compared to controls. Several additional types of neoplasms were less frequent among treated than control animals. This finding of negative dose trends related to a reduction in body weight, especially at the highest dosage of triprolidine, supports the findings of others who have noted direct correlations between body weight and tumor frequency (Albanes, 1987; Gries and Young, 1982; Haseman, 1983; Rao et al., 1987; Ross et al., 1970; Thurman et al., 1993). Thus, negative responses associated with triprolidine exposure probably were a consequence of body weight inhibition rather than a direct effect of the antihistamine.

It is clear from both rats and mice (Greenman et al., 1995b) that the parotid gland is a target organ for triprolidine. Cytoplasmic alterations of the parotid gland also have been reported in studies of other antihistamines in both species. They have been noted in chronic studies of doxylamine succinate in rats and mice (Jackson and Blackwell, 1993; Jackson and Sheldon, 1993) and in subchronic (Allaben, 1984) but not chronic studies of pyrilamine maleate in rats (Greenman et al., 1995a). It has been suggested that this morphologic response is related to the physiologic nature of the parotid gland and its pharmacologic response to antihistamines (Jackson and Blackwell, 1993). However, the biologic significance of the change still has not been resolved, and it is not yet clear whether to consider it of only physiologic interest or of potential pathologic concern in the assessment of human risk.

As with the parotid gland, liver is a target organ of triprolidine in both rats and mice (Greenman et al., 1995b). Signs of liver toxicity in rats were evidenced mainly by the presence of centrilobular fatty change and the increased incidence of chronic inflammation. However, there were also increases in the number of cellular foci of alteration. These effects were noted in both sexes and were mainly apparent in high-dose animals with some suggestion of effect at the mid-dose level. Since Harada et al. (1990) have reported an inverse relationship between leukemia and the presence of...
altered foci of the liver with a lowered frequency of foci in leukemic rats, we examined the data for a similar inverse relationship. Only three rats with altered foci also had leukemia. Thus, this inverse relationship was confirmed, but the positive trend in the incidence of altered foci was still clearly present in rats without leukemia. It is, therefore, apparent that the increased frequency of altered foci at the high triprolidine dose was not a consequence of a reduction in the frequency of leukemia in these animals. There was some evidence of changes at all dose levels in rats. Since focal changes noted in this study are often associated with the hepatocarcinogenic process and are considered by some to be preneoplastic changes (Harada et al., 1990), it cannot be ruled out that extension of the exposure period may have eventually resulted in liver neoplasia. Neither can it be excluded that failure to see heptocarcinogenesis at the highest dosage was related to body weight inhibition.

In conclusion, the only potentially toxic response noted in the studies reported here were exerted by triprolidine on the liver at dietary levels of 1000 ppm or above. This response was noted at a daily dosage over 200-fold higher than that of a 70-kg human. There was no clear evidence for a neoplastic response to triprolidine in F344 rats.

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