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TYROSINE Special Study

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DATA EVALUATION REPORT

STUDY TYPE: Special Study/Rats and Mice
Nonguideline

DP BARCODE: D224202
P.C. CODE: 123000
MRID NUMBER: 43904816

SUBMISSION NO.: S501233
TOX. CHEM. NO.: New Chemical

TEST MATERIAL (PURITY): Tyrosine (98%)

CITATION: Esdaile, D. J. 1995. Tyrosine: Exploratory 14-Day (Ocular Toxicity) Study in the Rat and Mouse. Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. SA 94100. December, 20, 1995. MRID # 43904816. Unpublished.

EXECUTIVE SUMMARY: In this exploratory study (MRID # 43904816), groups of 5 male and 5 female CD rats, Brown Norway rats and CD-1 mice received 0, 2 or 5% tyrosine (0, 1,000 or 2,500 mg/kg/day for rats and 0, 2,600 or 6,500 mg/kg/day for mice, respectively) in their diet for 14 days.

Within 48 hours of dietary administration of 5% tyrosine, corneal opacities with superficial keratitis were observed in 3 of 5 male CD rats; by Day 7, corneal opacities developed in all five rats. At study termination, these corneal lesions were found to be associated with elevated plasma tyrosine levels. One of five male Brown Norway rats receiving 5% tyrosine had slight bilateral opacities at 14 days accompanied by a high plasma tyrosine level. Histopathology revealed changes characteristic of corneal opacity involving various corneal layers and ciliary processes. These effects were not seen in female rats or mice of either sex. Dietary administration of 2% tyrosine failed to produce similar effects in any group or in any female rats and both sexes of mice. There were no differences between the control and treated groups in any of the other parameters measured.

The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS

A. Test Material

Name: L-tyrosine
Purity: 98%
Batch Number: 68160-123
Description: White crystalline powder
Storage Conditions: In an air-tight, light-resistent container at approximately 5°C

B. Vehicle: Basal diet

C. Test Animals:

Species: Rat and mice
Strains: CD Rats (Crl: CD (SD) BR; Brown Norway Rats (BN/Crl BR); CD-1 Mice (Crl: CD-1 (ICR) BR
Source: Charles River France
Age: Males and Females - 5 weeks at arrival
Weight: Males - CD Rats: 180.7-201; Brown Norway Rats: 103.4-112.4; CD-1 Mice: 27.0-29.7
Females - CD Rats: 168.1-187.8; Brown Norway Rats: 89.5-105.3; CD-1 Mice: 22.6-25.2
Housing: Individually in stainless steel cages
Environmental Conditions:
Temperature: 22 ± 2°C; Relative humidity: 55 ± 15%
Photoperiod: 12 hours light/dark
Air changes: 10-15/hour
Food and Water: Laboratory Rodent Diet powder A04C P1 (UAR, Villemoisson-sur-Orge, France) and tap water *ad libitum*
Acclimation Period: 6 days

II. METHODS

A. Preparation of Dosing Substance

Prior to initiation of the study, the test substance was incorporated into the basal diet by dry mixing to produce desired dietary concentrations. Dietary levels of the test substance were verified for each concentration. The homogeneity of tyrosine in test diets was verified. The stability of the frozen dietary mixtures were analyzed at study termination.

B. Dosage and Administration

Dosage Groups[a]

The animals were assigned to the following treatment groups using a randomization procedure based on body weight:

Test Group	Dietary Dose of Tyrosine (%)	# Males	# Females
CD Rats: 1	0	5	5
2	2	5	5
3	5	5	5
Brown Norway Rats: 4	0	5	5
5	2	5	5
6	5	5	5
CD-1 Mice: 7	0	5	5
8	2	5	5
9	5	5	5

[a] Dosages were selected by the sponsor; no rationale was provided.

Administration

An ophthalmological examination was conducted on all animals prior to study start. Animals were fed the respective test diets for 14 days; controls received basal diet for the same period. All animals were sacrificed after 14 days of treatment.

C. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies:

Clinical signs, mortality and morbidity - twice daily and once daily on

week-ends and holidays

Body weights - prior to treatment, on Day 1 and weekly thereafter
Food consumption - weekly intervals during the treatment period
Ophthalmoscopy - prior to treatment and on Days 2, 3, 7, 8 and 14 using indirect ophthalmoscope
Clinical chemistry- blood samples before necropsy
Gross necropsy - all animals
Histopathology - Left eye of selected animals

D. Plasma Tyrosine Analysis

At necropsy, blood was drawn from the abdominal aorta and collected on heparin. Plasma was centrifuged and subjected to derivatization to allow ultraviolet detection of free tyrosine. Samples were separated by High Performance Liquid Chromatography. Plasma tyrosine analysis was conducted for the following groups: all male rats from groups 1 through 6, male mice from groups 7 and 8 as well as all female CD rats from groups 1 and 3. The selection of groups was made on the basis of the presence of eye lesions; the selected groups also included control groups and other treated groups where the comparison of data were useful.

On Day 15, all animals were sacrificed following intraperitoneal injection of pentobarbital. Necropsy was performed on all major organs, tissues and body cavities. Left and right eye with Harderian glands were fixed in Davidson's fixative. Based on the results of ophthalmoscopic examinations, left eye of selected animals were processed and stained with hematoxylin-eosin for microscopic examinations. The selected animals included first and third animals from the groups of five for all groups of males and from females from groups 1 and 3.

E. Statistical Analyses

The following parameters were analyzed using the procedures described below:

- Body weight, body weight gains, food consumption and plasma tyrosine -- Means and standard deviations for each sex for each group

- Body weight gain and food consumption - compared using Dunnett's test

III. RESULTS

A. Administered Dosage

The homogeneity and stability of tyrosine in test diets indicated that the values were within ±9% of the targeted doses (range: 100-109% and 99-109% of nominal, respectively). The concentration analysis of the 2% and 5% tyrosine containing diets revealed that the doses administered to each group were within ± 4% of the targeted doses (104% and 102%, respectively).

B. Mortality/Clinical Observations and Systemic Toxicity

There were no deaths during the study. No clinical signs of toxicity were observed in male and female CD rats at 2% or male and female Brown Norway rats and CD-1 mice at 2% and 5% tyrosine. The treatment-related findings observed in CD rats receiving 5% tyrosine consisted of dark urine in all males during second week of the study and in 3 of 5 females on Day 14. In addition, one male from 5% tyrosine group appeared thin and had severe corneal opacity. This animal exhibited ptosis and piloerection from Days 10 and 13, respectively.

C. Body Weight and Body Weight Gain

Body weights and body weight gains of the treated animals did not significantly differ from those of the controls.

D. Food Consumption

There were no significant differences between the treated and control groups in mean daily food consumption.

E. Ophthalmoscopy

No treatment-related ophthalmoscopic changes were seen in female CD or Brown Norway rats at 2% or 5%, male CD and Brown-Norway rats at 2% and in either sex of mice at 2% or 5% tyrosine. Treatment-related ophthalmoscopic changes were limited to 5 male CD rats and one male Brown-Norway rat given 5% tyrosine diet.

The ophthalmoscopic findings are presented in Table 1. Treatment-related corneal opacities developed during Day 2 to 14 of treatment in all 5 male CD rats receiving 5% tyrosine. These opacities progressed from slight to severe and by Day 14, two males developed a very severe opacity and exhibited signs of edema and vascularization of the cornea; congestion of the iris was evident in three males. The corneal opacity was either focal or consisted of multifocal areas with each lesion having a snow-flake appearance. On Day 14, in more severe cases, edema and vascularization of whole cornea was noted. Among Brown Norway rats, only one male at 5% tyrosine developed slight opacity late in the study, on Day 14. There were no treatment-related corneal effects observed in male rats at 2% tyrosine and female rats at 2% or 5% tyrosine. None of the mice of either sex were affected at any dose.

Table 1
Ophthalmoscopic Findings in Male Rats fed 5% Tyrosine in Diet for 14 Days[a]

Species	Ocular Findings		
	Corneal Opacity	Other Corneal Effects	Congestion of Iris
CD Rat:			
#1380	Severe; bilateral	--	Mild
#1381	Very severe; bilateral	Edema and Vascularization	Severe
#1382	Very severe; bilateral	Edema and Vascularization	Severe
#1383	Severe; bilateral	--	--
#1384	Slight; bilateral	--	--
Brown Norway Rat:			
#1412b	Slight; bilateral	--	--

a Extracted from Table 5, page 60 the study report; the results reported in the table are for Day 14 of the study.
b This rat had a very high level of plasma tyrosine level (566 mg/L)

F. Plasma Tyrosine Analysis

The results of analysis are summarized in Table 2. Treatment-related increase in plasma tyrosine levels over base levels was noted in male (3 and 5 fold at 2% and 5% tyrosine, respectively) and female (5 fold at 5% tyrosine) CD rats. However, the corneal lesions were observed in males receiving 5% tyrosine. Similar increase in tyrosine level was noted

among Brown Norway male rats at 2% and 5% dietary tyrosine, but only one male from 5% group that developed corneal opacity had 10 fold increase in tyrosine level compared to other four males in the same group. The base levels of plasma tyrosine in CD male rats were higher than that of female CD rats and male Brown Norway rats and CD-1 mice. No treatment-related changes in plasma tyrosine levels were noted in male CD-1 mice.

Table 2
Mean Plasma Tyrosine Levels (mg/L) in Male Rats and Mice[a]

Species	Dosage Levels (%)		
	0	2	5
CD Rats	21 ± 4.2	59 ± 4.8	114 ± 39.9
Brown Norway Rats	12 ± 0.6	32 ± 15	68 ± 13.0b
CD-1 Mice	13 ± 1.8	- -	18 ± 7.1

a Extracted from Table 6, page 18 of the study report; for female CD rats the plasma tyrosine levels for the 0% and 5% tyrosine groups were 13 ± 2.0 and 62 ± 29.5 mg/l, respectively.
b Excludes one high outlier (tyrosine level = 566 mg/L)

G. Necropsy Findings

Gross Necropsy

There were no treatment-related changes on gross necropsy examination of the animals.

Histopathology

The histopathological findings are presented in Table 3. The histopathological examination of eyes revealed treatment-related changes in the corneal epithelium of two male CD rats from 5% dose group. These consisted of diffuse epithelial intracytoplasmic vacuolation, severe interstitial edema and swollen nuclear changes in the epithelial cells. In addition, inflammatory reaction involving the entire cornea was evidenced by diffuse polymorphonuclear (PMN) cell infiltration of stroma and epithelium, and focal infiltration of epithelium as well as ciliary

processes. These changes correlated with corneal opacities (superficial keratitis) observed during ophthalmoscopic examinations. These changes were not seen in female CD rats at any dose level or in male CD rats at 2% tyrosine. One of five Brown Norway male rats developed slight corneal opacity. Histopathology of the eye revealed changes including diffuse infiltration of the corneal stroma by PMNs with focal accumulation of PMNs in the anterior chamber and localized superficial epithelial desquamation of non-keratinized cells in the center of cornea. No treatment-related changes were observed in treated male mice.

IV. DISCUSSION/CONCLUSIONS

A. In this exploratory ocular toxicity study, groups of five male and five female CD rats, Brown Norway rats and CD-1 mice received tyrosine in diet at dose levels of 0%, 2% and 5% daily for 14 days. The high dietary intake of L-tyrosine caused increase in plasma tyrosine levels in rats but not in mice. These high tyrosine levels were found to be associated with the presence of corneal opacities. The dietary intake of tyrosine had no adverse effect on cornea in mice. CD rats were more susceptible to the corneal effects of tyrosine than the other strain of rat tested. Male CD rats had higher levels of tyrosine than female CD rats and male Brown Norway rats. Mice were resistent to effects of high dietary intake of tyrosine than rats. The histological findings of the affected eyes were characteristic of typical corneal lesions with a snow-flake appearance and were indicative of keratitis with an inflammatory reaction involving various corneal layers and ciliary processes. These lesions developed rapidly within 2-3 days of tyrosine intake and were multifocal.

The objective of this study was to ascertain the relationship between the high dietary intake of L-tyrosine and the presence of increased levels of plasma tyrosine in rats (but not in mice) as well as to establish a corelation between the presence of corneal opacities and high plasma tyrosine levels. The typical corneal lesions observed in the two strains of rats in this study were similar to those seen in other Acceptable, nonguideline study with tyrosine (MRID # 43904817).

B. Study Deficiencies: Histopathology of eye for the remaining male CD rats from 5% tyrosine group was not performed. However, this deficiency does not affect the outcome of the study results.

Table 3
Histopathology of Ocular Lesions in CD Rats Fed 5% Tyrosine in Diet for 14 Days[a]

Animal #	Ocular Findings in Various Regions of Eye					
	Cornea, Epithelium	Cornea, Subepithelium	Cornea, Endothelium	Cornea, Stroma	Anterior Chamber of Eye	Ciliary Processes
#1380	- Marked interstitial edema of the basal layer - cytoplasmic vacuolation and nuclear swelling/ edematous appearance of cells	- -	- Moderate to marked infiltration by PMNs - Vacuolation of endothelial cells	- Slight infiltration by PMNs	- -	- Minimal accumulation of PMNs at one pole of the eye
#1382	- Marked interstitial edema of the basal layer - cytoplasmic vacuolation and nuclear swelling/ edematous appearance of cells - slight infiltration by PMNs - focal hyperkeratosis	-Focal edema with PMNs infiltration	- Moderate to marked infiltration by PMNs - Vacuolation of endothelial cells	- Slight infiltration by PMNs	- -	- Severe accumulation of PMNs at one pole of the eye

a Extracted from Table 8, page 74 of the study report

Table 3 (Continued)
Histopathology of Ocular Lesions in Brown Norway Rats Fed 5% Tyrosine in Diet for 14 Days[a]

Animal #	Ocular Findings in Various Regions of Eye					
	Cornea, Epithelium	Cornea, Subepithelium	Cornea, Endothelium	Cornea, Stroma	Anterior Chamber of Eye	Ciliary Processes
#1402	-Focal solitary area of moderate vacuolation of cells[b]	--	--	--	--	--
#1412	-Focal superficial area of cellular desquamation of non-keratinized cells with underlying moderate accumulation of PMNs	--	--	- Mild, diffuse infiltration by PMNs at the base of the epithelium	- focal accumulation of PMNs at one pole	--

a Extracted from Table 8, page 76 of the study report
b On slide #15 only