

- **Negative Controls:** Solvent DMSO
- **Comments:** An individual stock solution/suspension of the test substance at a concentration of 50 mg/ml (plate assay) or 250 mg/ml (pre-incubation assay) was prepared for each experiment in DMSO and serial dilutions were carried out.
Dosing volume 100 µl/plate (except pre-incubation experiment – vol. Reduced to 20 µl/plate)
- **Doses used in definitive study:** Dose range of 5000 to 100 µg per plate, both in the presence and absence of S9-mix.
- **Study design:** Procedure followed the Ames protocol modified in accordance with the recommendations of the _____ sub-committee on guidelines for mutagenicity testing. The test substance was subsequently re-tested in all six strains over the same dose range; the +S9-mix phase of this second assay was conducted using a pre-incubation protocol. The incubation period for each experiment was 3 days at 37°C. For each experiment, positive control substances were tested to validate the bacterial strains and to confirm the activity of the S9-mix.
0.1 ml aliquots of a 10-12 hour culture of each strain were dispensed into the required number of containers, and stored at room temp. until required. Top agar consisted of 0.6% w/v agar and 0.5% w/v NaCl in deionized water, melted by brief _____ and stored at ca 50°C.
Prior to testing the molten top agar was prepared by adding sterile 0.5 mM histidine/0.5M biotin stock solution for Salmonella, and by adding a sterile tryptophan solution for E. coli.
0.5 ml S9-mix (or S9 buffer) was added to the number of aliquots required for one concentration, followed by 0.1 ml of the test substance. 2.0 ml top agar was added to each aliquot and poured onto the surface of a _____ plate Petri-dish prepared with 25 ml _____ minimal medium containing 1.5% w/v agar and 2% w/v glucose and allowed to gel. Plates were incubated at 37°C for 3 days in the dark.
[The pre-incubation protocol was slightly altered with regard to volumes and shaking.]
- **Analysis:**
- **No. slides/plates/replicates/animals analyzed:** Test Substance: 6 concentrations per strain; 3 plates per concentration. Two separate assays.
- **Counting method:** Automatic colony counter
- **Statistical methods:** One-tailed Student's t-test. The corresponding probability for each dose level was derived by computer using the appropriate degrees of freedom.
- **Criteria for Positive Results:** According to the Sponsor: A positive response is achieved when one or both of the following criteria are met:
 - a) a statistically significant dose-related increase in the mean number of revertant colonies is obtained;
 - b) a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) which is statistically significant, is observed at one or more concentrations.

Results: In two separate assays, the test substance did not induce any significant, reproducible increases in the observed number of revertant colonies in any of the tester strains used, in the presence or absence of S9-mix.

- **Study Validity:** Positive controls induced the expected responses.
- **Study Outcome:** Ames test was negative. Under the conditions of assay, NTBC Purified gave a negative, non-mutagenic response in S typhimurium strains TA1535, TA1537, TA98 and TA100 and E. coli strains WP2P and WP2P urvA in the presence and absence of S9-mix.

Summary: See Results section above and tables below.

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Sponsor's Table Vol. 1.6/187

TABLE 1 - TEST DATA FOR EXPERIMENTAL PHASE 1 (+S9)

Key to Statistical Significance: * : 0.01 ≤ P < 0.05, ** P < 0.01
One-sided t-Test assumes Test > Control

COMPOUND Y10506/001 NTBC		BATCH:10912/94 EX SWEDISH ORPHAN AB			DATE TESTED 10/05/99				
STUDY YV4404		TEST 00	SPONSOR	EXTERNAL SPONSOR	DATE COUNTED 13/05/99				
STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE			
						1	2	3	
TA 1535	+S9 5000	24.0	13.0	1.7		┌			
	2500	20.7	6.7	1.4					
	1000	11.3	2.1	0.8					
	500	13.3	5.0	0.9					
	200	14.7	1.5	1.0					
	100	7.3	2.9	0.5					
TA 1537	+S9 5000	17.7	28.0	1.7					
	2500	6.3	2.3	0.6					
	1000	8.0	3.0	0.8					
	500	13.0	2.6	1.3					
	200	10.3	7.1	1.0					
	100	11.0	1.7	1.1					
ZA 98	+S9 5000	10.3	3.1	0.4					
	2500	29.3	9.5	1.0					
	1000	35.0	5.6	1.3					
	500	35.3	8.0	1.3					
	200	34.0	11.3	1.2					
	100	31.7	4.2	1.1					
TA 100	+S9 5000	155.7	50.5	1.2					
	2500	111.7	18.5	0.9					
	1000	129.3	15.0	1.0					
	500	155.7	51.1	1.2					
	200	203.0	36.5	1.6					
	100	154.3	9.7	1.2					
WF2P	+S9 5000	41.3	13.6	0.8					
	2500	43.9	3.8	0.9					
	1000	30.3	5.5	0.6					
	500	31.0	11.1	0.6					
	200	36.0	15.0	0.7					
	100	34.0	9.5	0.7					
WF2P uvFA+S9	5000	191.0	20.1	1.1					
	2500	176.0	19.3	1.0					
	1000	126.7	8.4	0.7					
	500	142.7	24.1	0.8					
	200	131.7	15.0	0.7					
	100	111.0	25.1	0.6					

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TABLE 2 - TEST DATA FOR EXPERIMENTAL PHASE 1 (-S9)

COMPOUND Y10506/001 NTBC

BATCH:10912/94 EX SWEDISH ORPHAN AB
 STUDY IV4404 TEST 01 SPONSOR EXTERNAL SPONSOR

DATE TESTED 14/05/99
 DATE COUNTED 17/05/99

COMMENTS BACKGROUND LAWN ABSENT/SPARSE ON 5000/2500ug DOSE PLATES.

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE		
						PLATE 1	PLATE 2	PLATE 3
TA 1535	-S9 5000	0.0	0.0	0.0				
	2500	6.0	4.4	0.4				
	1000	5.7	3.2	0.4				
	500	10.7	3.1	0.8				
	200	8.3	0.6	0.7				
	100	12.3	0.6	0.9				
TA 1537	-S9 5000	0.0	0.0	0.0				
	2500	3.0	2.0	0.2				
	1000	13.3	5.7	1.0				
	500	10.0	2.0	0.8				
	200	15.3	4.5	1.2				
	100	11.3	3.1	0.9				
TA 98	-S9 5000	7.3	4.7	0.3				
	2500	15.0	5.0	0.7				
	1000	18.0	3.6	0.6				
	500	20.3	3.5	0.9				
	200	23.7	5.5	1.1				
	100	28.7	6.0	1.3				
TA 100	-S9 5000	34.7	15.7	0.2				
	2500	58.3	15.7	0.6				
	1000	124.3	51.4	0.9				
	500	190.0	61.4	0.8				
	200							
	100							
21 Key to Statistical Significance: * : 0.01 ≤ P < 0.05, **: P < 0.01 11 One-sided t-Test assumes Test > Control								
WP27	-S9 5000	31.0	4.0	0.8				
	2500	35.0	4.4	0.9				
	1000	45.7	8.1	1.2				
	500	36.0	6.9	1.0				
	200	46.0	6.1	1.2				
	100							
WP27 uvra-S9	5000	79.3	18.5	0.4				
	2500	205.3	59.1	1.1				
	1000	197.7	9.0	1.0				
	500	197.7	12.9	1.0				
	200	200.3	12.0	1.0				
	100	215.7	7.5	1.1				

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GENETIC TOXICOLOGY (Continued)

Study Title: NTBC UNPURIFIED: Bacterial Mutation Assay in S. Typhimurium and E.Coli
Study No: Report — /P/6301; — Study No. YV4405; Reference Nos. Sponsor: C08220;
— Test Subs. Y10506/002
Study Type: Ames - Bacterial Reverse Mutation Assay.
Volume # and Page #: Vol. 1.6 p 199
Conducting Laboratory: _____

Date of Study Initiation/completion: 06 May 99; experimental phase started 10 May 99;
completed 27 May 99. Report dtd. 27 Jul 99.

GLP Compliance: OECD Principles of Good Laboratory Practice (Ref. 471), revised 1997. (ENV/MC/CHEM(98)17). The stability, homogeneity and achieved concentration of the test substance and control substances in the vehicles used were not determined by analysis and certified purity and stability of the control substance was not available (by — ICH S2A and S2B

QA- Reports Yes (X) No ():

Drug Batch Number: 13781-50-01 Purity —

Comments:

This study was carried out by _____ at the same time and by the same methods and procedures as the genetic toxicology study on the NTBC Purified: Bacterial Mutation Assay in S. Typhimurium and E. coli above.

Results:

NTBC Unpurified: Two separate assays did not induce any reproducible statistically significant drug-related increases in the observed number of revertant colonies in the following strains: TA1535, TA100, WP2P, or WP2P uvrA with or without S9-mix or in TA1537 +S9-mix.

However, both experiments showed small increases in revertant colony numbers with TA98 with and without S9-mix and with TA1537 in the absence of S9-mix.

Positive controls gave the expected responses.

- **Study Validity:** Positive controls induced the expected responses for each experiment.

- **Study Outcome:** TA98 with and without S9-mix and TA1537 without S9-mix showed increases in revertant colony numbers.

Summary: See Results section above and tables below.

The Unpurified NTBC test substance showed evidence of a mutagenic response in strains TA1537 and TA98.

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Sponsor's Table Vol. 1.6/221

TABLE 1 - TEST DATA FOR EXPERIMENTAL PHASE 1 (+S9)

COMPOUND Y10506/002 NTBC

STUDY YV4405 TEST 00 UNPURIFIED MATERIAL EX SWEDISH ORPHAN, BATCH 13701-50-01
 SPONSOR EXTERNAL SPONSOR DATE TESTED 10/05/99
 DATE COUNTED 13/05/99

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE		
						1	2	3
TA 1535	+S9 5000	10.0	3.5	0.7				
	2500	13.0	2.6	0.9				
	1000	8.3	1.5	0.6				
	500	11.7	3.2	0.8				
	200	9.7	1.2	0.7				
	100	15.0	1.0	1.0				
TA 1537	+S9 5000	2.3	1.5	0.2				
	2500	10.0	2.0	1.0				
	1000	8.0	1.0	0.8				
	500	12.3	2.1	1.2				
	200	20.3	7.0	2.0	*			
	100	13.7	6.4	1.3				
TA 98	+S9 5000	21.7	6.5	0.8				
	2500	52.0	5.6	1.9	**			
	1000	43.7	3.5	1.6	**			
	500	35.7	10.1	1.3				
	200	31.3	4.7	1.1				
	100	33.7	11.0	1.2				
TA 100	+S9 5000	139.0	34.9	1.1				
	2500	178.3	6.4	1.4				
	1000	152.7	59.8	1.2				
	500	151.0	7.8	1.2				
	200	181.0	24.6	1.4				
	100	117.7	26.0	0.9				
WP2P	+S9 5000	35.3	8.1	0.7				
	2500	44.0	8.5	0.9				
	1000	22.3	7.3	0.4				
	500	33.7	13.7	0.7				
	200	39.7	1.2	0.8				
	100	30.3	6.0	0.6				
WP2P UVIA+S9	5000	219.7	36.7	1.2				
	2500	219.0	22.5	1.2				
	1000	150.0	25.0	0.8				
	500	158.0	31.3	0.9				
	200	157.0	21.7	0.9				
	100	140.0	25.5	0.8				

Key to Statistical Significance: * : 0.01 ≤ P < 0.05, ** P < 0.01
 One-sided t-Test assumes Test > Control

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TABLE 2 - TEST DATA FOR EXPERIMENTAL PHASE 1 (-S9)

Key to Statistical Significance: *: 0.01 ≤ P < 0.05, **: P < 0.01
One-sided t-Test assumes Test > Control

COMPOUND Y10506/002 NTBC

UNPURIFIED MATERIAL EX SWEDISH ORPHAM, BATCH 13781-50-01
STUDY YV4405 TEST 01 SPONSOR EXTERNAL SPONSOR DATE TESTED 14/05/99
DATE COUNTED 17/05/99

COMMENTS BACKGROUND LAWN ARSENT/SPARSE ON 5000ug DOSE PLATES.

STRAIN	DOSE LEVELS (MICROGRAMS/PLATE)	MEAN	STANDARD DEVIATION	RATIO: TEST/CONTROL	STATS SIGNIF.	NO REVERTANTS/PLATE		
						PLATE 1	PLATE 2	PLATE 3
TA 1535	-S9 5000	0.0	0.0	0.0				
	2500	3.7	1.2	0.3				
	1000	9.7	3.8	0.7				
	500	8.3	4.0	0.6				
	200	12.7	3.2	0.9				
	100	11.3	3.5	0.8				
TA 1537	-S9 5000	1.0	1.0	0.1				
	2500	11.3	4.2	0.9				
	1000	24.7	5.0	1.9	**			
	500	17.0	7.2	1.3				
	200	11.3	7.4	0.9				
	100	12.3	1.5	0.9				
TA 98	-S9 5000	16.3	4.9	0.8				
	2500	32.7	4.7	1.5	**			
	1000	47.3	7.2	2.2	**			
	500	28.0	3.0	1.3	*			
	200	29.7	4.0	1.4	*			
	100	24.0	2.6	1.1				
TA 100	-S9 5000	51.3	10.7	0.4				
	2500	96.0	15.7	0.7				
	1000	91.3	11.0	0.6				
	500	57.0	10.4	0.4				
	200	88.7	17.5	0.6				
	100	78.3	19.4	0.5				
WP2P	-S9 5000	14.0	1.7	0.4				
	2500	33.3	5.9	0.9				
	1000	32.7	2.5	0.9				
	500	34.3	3.8	0.9				
	200	28.0	4.4	0.8				
	100	33.0	6.1	0.9				
WP2P UV2A-S9	5000	85.7	23.2	0.4				
	2500	216.0	7.9	1.1				
	1000	214.3	49.3	1.1				
	500	199.0	10.5	1.0				
	200	173.0	4.4	0.9				
	100	189.0	21.8	1.0				

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Study T-12611: MORPHOLOGICAL TRANSFORMATION OF BALB/3TC CELLS: Vol. 1.6 p. 292
Reference IND — Review dtd 26 Jan 95 p.11 attached.

As reported previously in IND — SC-0735, Technical had no transforming activity when tested without activation under a dose range of 0.0125 to 0.200 mg/ml.

Results in the present report T-12611, Project 140284, Report 8804-12, (Lot — -9703-48-1 Laboratory: —) dtd 12 Apr 88 indicate that in the presence of an activation system from Aroclor 1254 induced rat liver, there was no transforming activity at doses up to 1.75 mg/ml in DMSO under the conditions of the assay. [BALB/3T3 cells usually cease cell division upon forming a confluent monolayer. Transformed cells lose this contact inhibition and continue to proliferate.]

The sponsor indicates that the difference in relative toxicity between the direct assay and the activation system may reflect the difference in treatment times rather than an effect of the activation system. In the direct assay, cells are tested for 3 days, while the use of an activation system requires that treatment be limited to 4 hours. SC-0735 Technical is insoluble in DMSO at concentrations greater than 200 mg/ml, and insoluble in medium with activation at 2.00 mg/ml precluding testing with higher concentrations.

[Study (initiated 03/11/86, 06/26/86) was originally submitted to the U.S. Environmental Protection Agency in general in compliance with EPA FIFRA GLP Standards. QA statement present.]

Comments:

The sponsor states that for freely soluble substances that are non toxic, an upper dose range of 3 to 10 mg/ml is usually established and that other compounds must be tested either to the limits of solubility or to a less than 50% relative clonal survival. It would appear that since SC-0735 with activation is insoluble at 2.0 mg/ml these results might be uncertain.

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SPECIAL STUDIES/OTHER INFORMATION

Effects of Compound 0735 on Brain (Striatal Region) Biogenic Amino Levels:

[The compound is unlikely to be a tyrosine hydroxylase inhibitor (in brain), since under both acute and repeated administration, it did not lower catecholamine levels (i.e., dopamine or norepinephrine). Typically, α -methyl tyrosine, dosed via a similar route of administration at 50 or 100 mg/kg, would cause a greater than 50% reduction in catecholamine levels.]

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Sponsor's Table Vol. 1.7/096

Vehicle = cornoil. Male Rats.

ACUTE AND SUBACUTE EFFECTS OF
COMPOUND 0735
ON BRAIN (STRIATAL REGION) BIOGENIC AMINO LEVELS

<u>Treatment</u>	<u>(N)</u>	<u>DA</u>	<u>DOPAC</u>	<u>HVA</u>	<u>5HT</u>	<u>5HIAA</u>	<u>NE</u>
			($\mu\text{g/g}$)	(S.E.)			
Chronic vehicle	(8)	10.65 \pm 0.28	1.04 \pm 0.14	0.68 \pm 0.03	0.43 \pm 0.01	0.509 \pm 0.02	0.25 \pm 0.01
Acute with compound 0735 (10 mg/kg, p.o.)	(7)	8.86 \pm 0.20*	1.01 \pm 0.05	0.54 \pm 0.09	0.43 \pm 0.02	0.53 \pm 0.04	0.31 \pm 0.02
Subacute with compound 0735 (10 mg/kg, p.o. 2 weeks)	(7)	10.05 \pm 0.74	1.48 \pm 0.09*	0.98 \pm 0.07*	0.412 \pm 0.02	0.45 \pm 0.02	0.31 \pm 0.06

*Significantly different from control $p < 0.5$

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Overall Summary:**Preclinical Studies:**

For completeness, studies from the IND (Review dtd. 26 Jan 95 – attached) and those reviewed in this NDA have been summarized below. [See Table of Contents for review references.] T-numbers are study numbers.

Preclinical studies with radioactive SC-0735 (NTCB) have shown selective retention of radiolabel in the liver and kidneys and to a lesser extent in the Harderian gland. Single oral doses of 0.1 or 10 mg/kg in the rat have shown no retention of radiolabel in the cornea, the site of toxicity. There was however, a marked increase of tyrosine concentration in both plasma and ocular fluid. SC-0735 appears to bind to a protein or proteins in liver cytosol, leading to its retention, which is associated with inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase that causes a marked and sustained tyrosinemia. SC-0735 is also retained in mouse liver but to a lesser extent than in the rat liver.

It is reported that pharmacological evaluation has shown no significant effects on heart rate, blood pressure, cardiac force, QA interval or respiration rate and no evidence of effect at A1, A2, or B2 adrenoreceptors. High oral doses in rats (500 mg/kg) possibly reflecting a toxic response were prolonged halothane induced sleeping time, prolonged "pull-up" times and clinical signs indicative of generalized systemic toxicity.

NTCB appeared to be fairly rapidly and completely absorbed after oral administration. The terminal half-life following an intravenous injection in rats was ca. 9.3 hours. When fitted to a one compartment model, an oral suspension of 2.61 mg/kg gave a mean C_{max} of 6.57 $\mu\text{g/mL}$ and a mean t_{max} of 5.49 h. The bioavailability was 90.6%. An oral solution of 3.03 mg/kg gave a bioavailability of 101% with a mean C_{max} of 10.10 $\mu\text{g/mL}$ and a mean t_{max} of 3.57h. There was no apparent difference in dose normalized AUC values from rats treated with the oral suspension or oral solution and intravenous injections. [These data do not fulfill that requested in Pharm. Review of IND (Review dtd. 26 Jan 95.) [For a limited summary comparing a capsule and liquid formulation in humans see p. 11.]

Male and female rats were sacrificed 6 hours after a single oral dose of 100 mg/kg ^{14}C [Benzene ring]-SC-0735 or ^{14}C -[Dione ring]-SC-0735. Recovery averaged ca. 87.3% for the benzene ring labeled compound and 104.1% after the dione ring labeled SC-0735. At this time period, 3.3-6.7% of the radiolabeled dose was found in the urine. Internal organs showed 22.5-39.9%, carcass 27.7-43%, skin 11.4-14.4% and blood 4.5-9.1%.

Tear-forming tissues of the eye (tears possibly being a major route of corneal exposure) showed higher concentrations than the vitreous humor or lens. It is reported that only 1.2-2.3% of urinary radioactivity co-chromatographed with the ^{14}C -SC-0735 standard vs. 40.1% for the eye.

Toxicity Studies:**Rats**

An intended 13-week subchronic and two-year chronic toxicity/oncogenicity study (T-12984) in rats with doses of 0, 1, 5, 40, 120, 320, 800 ppm (ca. 0, 0.05, 0.25, 2, 6, 16, and 40 mg/kg) SC-0735 was aborted at 12 months when it was decided not to develop SC-0735 further. After 12 months, 10 rats per sex per dose group were necropsied and (most) organs subjected to histopathology. In-life data after 3 and 12 months showed mainly corneal lesions previously seen. Liver and kidney weights showed some increases, mainly at three months. At 12 months histopathological examination of the majority of tissues showed mainly a dose related corneal keratitis affecting all dose groups. Females at 800 ppm showed a slight liver centrilobular hypertrophy. Other non-neoplastic findings and incidental tumors did not appear to be treatment-related.

At study (T-12984) termination, 45 SC-0735 treated rats with ocular lesions were maintained for a further 3 months (T-13249) on control diet to observe whether corneal lesions would regress. Capillary vessels remained but were devoid of blood after the induced vascularization subsided; thus, rats with ocular lesions did not fully recover after 13 weeks on basal diet.

A 3-month study (T-13255) was carried out in rats by different routes (IND Review p. 6) to determine ocular changes at doses of 1 mg/kg by gavage, i.p., s.c. and i.v. 1x/day or 20 µg 2x/day ocular, or 20 ppm in the feed. The in-life portion of the study was stopped after 2 months when 25% of the s.c. rats developed significant corneal opacities with vascularization compared to 5% i.v., 10% in the diet, 10% i.p., and 15% p.o.

Mice

A 6-week dietary probe (T-12963) was carried out in mice (IND Review p. 7) at doses of 0, 300, 1000, 3000, 7000 and 10,000 ppm in the diet (ca. 0, 60, 200, 600, 1400 and 2000 mg/kg). The three higher doses produced body weight reductions and treatment changes, adaptive in liver, and degeneration in peripheral nerves. Females appeared to be more sensitive than males.

A planned dietary carcinogenicity study in mice (T-13005) of both sexes (IND Review p. 7) at doses of 0, 10, 350, 1500, 3000 ppm (ca. 0, 2, 70, 300, 700 mg/kg) that was terminated after 6.5 months did not appear to produce increased treatment related histopathology in the eyes of 10 animals/sex/group. Other tissues were not examined histopathologically. [The drug has produced corneal lesions within weeks of administration in rats – there was a possible mixing of species in IND Review p. 7.]

Dogs

Beagle dogs received SC-0735 (T-12965) orally at doses of 0, 1, 5, 10, 50, 100 and 150 mg/kg for 4 weeks in a range finding study. Treatment-related mortality and neurological abnormalities (without microscopic lesions) were seen at doses of 10 mg/kg and above. Lenticular lesions seen at 10 mg/kg were not confirmed microscopically. Ataxia and corneal alterations were present at 5 mg/kg; target organ toxicity at 1 mg/kg was limited to corneal alterations. Emaciation, reduced body weight gain and GI irritation/hemorrhage, were also evident. There were no clear sex toxicity differences.

A 3 month study (T-13004) was originally planned in groups of 4 Beagles/sex/group at dose levels of 0, 0.1, 0.5, 1.5 or 5 mg/kg/day. Corneal opacities were noted at 0.1 mg/kg (week 7 – first examination period); thus, a no-effect-level could not be ascertained and the study was extended to Week 32 to assess reversibility.

Corneal lesions were characterized by disorganization of the epithelium, loss of stratification, an increase in epidermal cell turnover, intracellular vacuoles, necrotic and inflammatory cells, keratohyaline granules, differences in cellular electron densities and changes in the epithelial membrane.

Some of the opacities disappeared or were reduced by Week 32 following dose discontinuation at Week 23, suggesting some reversibility [opacities no longer apparent in 4/4 in which dosing was discontinued and in 2/4 in which dosing was continued at original levels to Week 32].

Rabbits

A 3-month ocular toxicity probe of NTBC (T-13256) was carried out orally in rabbits at doses of 50 or 250 mg/kg. All of the high dose rabbits died the first week of study. Three of four 50 mg/kg rabbits died days 14, 23, and 43. The last rabbit was sacrificed on day 102. None of the treated rabbits showed corneal opacities.

Monkeys

A toxicity study (T-13587) was carried out in 2/group male rhesus monkeys at oral doses (5 days/week) up to 10 mg/kg for 2 weeks followed by a 2-week recovery period and then 13 weeks of dosing of the 10 mg/kg group followed by another recovery period with clinical pathology evaluation. No general or ocular toxicity was noted except for one animal that had a significant reduction in weight and showed a small linear corneal opacity near the canthus at a dose of 10 mg/kg.

Reproduction Studies:

Rats

A fertility probe (T-12725) was carried out in mature CD rats in which males were treated 60 days prior to mating and females 14 days prior to mating through postpartum day 21 with 100 mg/kg NTBC orally by gavage. Paternal toxicity included reduced body weight and food consumption. One treated male had eye opacities. Maternal toxicity included reduction in body weight and food consumption and absolute heart and ovary weights. Litter size was reduced and there was a decrease in the percent survival of live born pups and mean pup weight at birth.

A teratology screen (T-12724) was conducted with NTBC in CD rats at doses of 0, 200 mg/kg day 8-15 of gestation. Maternal and embryofetal toxicity was evident. There were no teratogenic effects.

A rangefinding teratology probe (T12935) was carried out in CD rats with SC-0735, Technical at doses of 0, 20, 50, 125 mg/kg administered days 8-20 of pregnancy. Maternal body weights and weight gains were reduced. Relative (but not absolute) kidney weight increases were statistically significant at 125 mg/kg.

Embryofetal toxicity was seen at the two higher doses. Mean pup weight, mean change in pup weight for postpartum days 0-4 and percent of pup viability were also significantly reduced at 125 mg/kg (1/3 were stillborn at 125 mg/kg).

There was no evidence of teratology at any dose level.

A cross-fostering reproduction study (T-13247) was carried out in rats treated with 100 mg/kg SC-0735. Maternal toxicity consisted of reduced body weight, body weight gain and food consumption, an increased incidence of corneal opacities and increased relative liver and kidney weights. Intrauterine exposure resulted in reduced pup survival, mean pup weight and delayed eye opening. Lactational exposure reduced mean pup weight and produced corneal opacities in weanlings. Lens lesions were produced in rats with prior intrauterine drug exposure regardless of whether or not they were exposed to drug in the diet during the 8 wk. postweaning phase. Corneal lesions were produced in rats exposed to drug during the postweaning phase regardless of whether or not they were exposed prenatally.

Mutagenicity Studies:

NOTE: According to the reviewing Chemist, the compound presently used clinically is a purified compound. The NTBC unpurified and SC-0735, Technical compounds would appear to contain (unnamed) impurities which might account for the (+) findings in certain of the mutagenicity tests below.

A number of studies (IND Review pgs. 10-12 and NDA Review p. 31) were carried out with SC-0735 to investigate mutagenicity. These included:

Ames Tests: Salmonella – Negative (T-12608) SC-0735, Technical (Lot — 9703-48-1)

Salmonella and E. coli – NTBC purified: Negative (Report — /P/6300)

(Batch 10912/94)

Salmonella and E. coli – NTBC unpurified: (Report — /P/6301)

(Batch 13781-50-01)

Small increases in revertant colony numbers were seen with strain

TA98 in the presence and absence of S9-mix, and with strain

TA1537 in the absence of S9.

Mouse Lymphoma multiple endpoint Forward Mutation Assay: (T-12609)

SC-0735, Technical (Lot — 9703-48-1) (unpurified) induced a dose dependent mutagenic response (>2.5 fold background) at doses of 0.300 mg/ml to 0.500 mg/ml.

Mouse lymphoma multiple endpoint test - Cytogenic assay: (T-12610)

SC-0735, Technical (Lot — 9703-48-1) (unpurified)

Doses from 0.0625 to 1.000 mg/ml without activation reduced survival to about 20%, but did not induce significant levels of aberrations or SCE's (sister chromatid exchanges).

A dose range of 0.0625-0.5000 mg/ml was evaluated with Aroclor rat liver activation. The highest doses produced a dose-related increase in aberrations and a significant increase in SCE at all doses evaluated – considered clastogenic by the Sponsor under the conditions of assay.

Morphological transformation of BALB/3T3 cells: (T-12611)

SC-0735, Technical (Lot 9703-48-1) (unpurified)

SC-0735 showed no transforming activity without activation at 0.125-0.200 mg/ml or in the presence of Aroclor induced rat liver at doses up to 1.75 mg/ml.

Effects of NTBC on Human Fibroblast DNA: (T-12664)

SC-0735 (Lot 9703-48-1) (Technical?, unpurified)

SC-075 did not induce significant strand breaks or repair in DNA at exposures of 1 mg/ml for 1 hour.

Mutagenicity Evaluation in Bone Marrow Micronucleus in Mice: (T-12771)

SC-0735, Technical (Lot — 9703-48-1) (unpurified)

NTBC was given to mice at doses of 0, 125, 250, 500, 600, 800, 1000 and 1200 mg/kg orally. There were deaths in males and females at 500 mg/kg. The number of polychromatic erythrocytes per 1000 erythrocytes was not statistically different from the control in either males or females indicating a lack of clastogenicity.

Special Studies:

A number of special studies (IND Review pgs. 12-14) were also carried out to further investigate ocular changes.

When rats were given NTBC orally (Report — 'R/1192) at doses of 0, 2, 10 or 40 mg/kg for 21 weeks corneal opacities with or without vascularization developed between 2 and 14 weeks. The maximum incidence (18/20) was seen at 2 mg/kg/day thus, dosing of half of the rats in this group was discontinued (for recovery) week 14 until termination at week 21. Changes including keratitis with polymorphonuclear leukocyte and eosinophil infiltration were partially reversible at the 14-week drug withdrawal. By week 21 the only ophthalmoscopic change present was corneal ghost blood vessels in some animals.

Seventy male, 35 day old rats were dosed orally (Report — 'R/1198) with 10 mg/kg/day. Rats with corneal lesions were removed from the study at intervals so that a range of lesions, from one day old to several days old, could be investigated. Light microscopy showed 48 of these developed corneal lesions consisting of focal areas of opacity. Rats with corneal lesions were removed. Change to a low protein diet following several weeks of dosing did not significantly change the incidence of rats with corneal lesions.

The effects of L-tyrosine supplemented low protein diet were studied (Report — 'R/1201) in the rat. High plasma tyrosine levels have been reported to be induced by a 5% L-tyrosine supplemented low protein diet in the rat. Weanling rats fed 5% L-tyrosine and a low protein diet showed a markedly elevated plasma tyrosine (2786 vs. 182 nmol/ml) and from day 1 they developed multifocal areas of corneal opacity which became confluent with time. Ophthalmoscopic and ocular histological findings in these rats are reported to resemble those induced by SC-0735 although they were more severe and rapidly occurring suggesting the possibility that ocular toxicity of SC-0735 is due to the raised plasma tyrosine levels rather than direct drug toxicity.

NTBC-induced tyrosinemia was studied (Report — 'R/1206) in the dog, rabbit and rhesus monkey.

Single oral doses of 10 mg/kg SC-0735 (30 μ mol/kg) to either New Zealand rabbits or Beagle dogs produced a marked elevation of tyrosine in both plasma and ocular fluid at which time 4-hydroxyphenylpyruvate dioxygenase activity was markedly inhibited.

basis in a 6.5 month study at 700 mg/kg and 120 times the maximum child dose in a 6 week study at 2000 mg/kg.

In addition to known ocular, liver and kidney system problems is the finding of limb weakness in mice and dogs that may be symptomatic of nervous/muscle system disorders.

A combination 3-month subchronic toxicity and 2 year chronic toxicity/oncogenicity study of SC-0735 administered in the diet at doses from ca. 0.05 up to 40 mg/kg was aborted at 12 months when development plans for the original intended use were cancelled. [This study was not pre-approved by FDA.] When plans for development of SC-0735 were cancelled it was decided to modify the study and only perform the scheduled necropsy after 12 months on 10 animals of each sex and sacrifice the remaining except for a few to be used for a recovery phase.

As expected, eyes were affected showing a dose related corneal keratitis in all dose groups. (Report T-13249 - 88-06-27 IND Review p. 6 indicated little or no ocular lesion recovery).

Body weights showed a decrease above 2 mg/kg. There were also some slight but statistically significant changes in hematological parameters and decreases in high dose blood glucose and a slight decrease in serum potassium of the two high dose female groups.

Liver and kidney weights showed some increases in weight in males without apparent histopathology; there was some centrilobular hypertrophy in high dose females. Liver and kidney changes would not be unexpected.

At this point the overall incidence of tumors was low and not considered treatment related. However, due to the reduced number of animals and the reduced time of exposure the response to SC-0735 carries little or no weight as a carcinogenicity study.

A Fertility probe carried out in rats at a single dose of 100 mg/kg (12 times the maximum child dose of 1 mg/kg b.i.d. on a surface area basis) produced a reduced litter size, a decrease in percent survival of live born pups and mean pup weight at birth. Since this was a single dose study, it is not known if lower doses would also produce the same effect; however this is basically a drug for use in young children in a life threatening situation.

A teratology screen was conducted with NTBC in rats. There were no teratogenic effects however, this screen was carried out at only one dose, 200 mg/kg (24 times the maximum child dose of 1 mg/kg b.i.d. on a surface area basis).

A rangefinding teratology probe was carried out in CD rats with SC-0735, Technical at doses of 0, 20, 50, 125 mg/kg administered days 8-20 of pregnancy in order to determine dose levels for a definitive teratology study in rats. Body weights and weight gains were reduced. Relative (but not absolute) kidney weight increases were statistically significant at 125 mg/kg.

Embryofetal toxicity was seen at the two higher doses. Mean pup weight, mean change in pup weight for postpartum days 0-4 and percent of pup viability were also significantly reduced at 125 mg/kg (1/3 were stillborn at 125 mg/kg).

There was no evidence of teratology at any dose level.

A definitive teratology study was not carried out in rats.

A cross fostering study in rats was also carried out with only one dose, 100 mg/kg (12 times the maximum child dose of 1 mg/kg b.i.d. on a surface area basis). This was not a reproductive study but an investigation of the production of eye lesions. Lens lesions were produced in rats with prior intrauterine drug exposure regardless of whether or not they were exposed to drug in the diet during the 8 week postweaning phase; corneal lesions were produced in rats exposed to drug during the postweaning phase regardless of whether or not they were exposed prenatally.

Although no teratology was seen in preliminary studies, the teratology and cross fostering studies do not qualify according to our guidelines for inclusion in the Pregnancy section of the labeling.

Positive findings were seen in some tests for mutagenicity. In both the direct and activation assays of the mouse lymphoma forward mutation test there was a significant dose-related increase in mutant frequency at doses of 0.300 mg/ml or greater. It also appeared that NTCB was clastogenic

and an inducer of sister chromatid exchange when tested in the presence of an Aroclor induced rat liver activation system in the mouse lymphoma cytogenic assay. Both of these studies were carried out with Technical grade (unpurified) SC-0735 that may have contained un-named impurities. In addition small increases in revertant colony numbers were seen with an unpurified batch of NTBC in the Ames test with strain TA98 in the presence and absence of S9-mix, and with strain TA1537 in the absence of S9. There is no apparent explanation for the negative findings in an apparently unpurified batch of NTBC in another Ames assay; however, there was a dose dependent response in strain TA98 with S9 (<2.5 fold over background)

An Ames test with a purified NTBC such as that used clinically was negative. Thus, it would appear that the NTBC unpurified and SC-075, Technical (unpurified) contain some (un-named) impurities which might account for the (+) findings in certain of the mutagenicity tests above. According to the reviewing Chemist, the compound presently used clinically is a purified compound and he did not know the identity of possible impurities in the original compound.

In addition, it would appear that since SC-0735 with activation was insoluble at 2.0 mg/ml in the morphological transformation of BALB/3TC cells test, results might be uncertain (see p. 38).

The original preclinical toxicology program was designed to comply with the use of NTBC as a

Thus, these studies are somewhat limited and do not always follow the guidelines for customary NDA support. In addition, for some of the raw data available, the quality of the printed documents was very poor and difficult to read. Dr. Ronald Leonardi, Regulatory Consultant, Swedish Orphan, at the 17 Dec 98 meeting indicated that toxicology studies were performed in compliance with GLP's and not as was stated in their submission letter (20 Nov 98?). [GLP compliance was not always stated with each individual study.]

For the specific Orphan Drug indication of Hereditary Tyrosinemia Type 1 (HT-1), a potentially fatal disease, the Division of Metabolic and Endocrine Drug Products indicated that it would be willing to work with the (preclinical) information the sponsor has available; however, eye and liver toxicities were of concern and should be addressed in an animal toxicity section included in the label (IND pre-NDA meeting of 17 Dec 98 – Dr. Ronald Steigerwalt, Pharmacology Team Leader, DMEDP).

It was concluded at this meeting that the overall effect of NTBC treatment on the disease (liver disease and death) out weigh the eye problems. [Should the sponsor desire any other indication for NTBC, additional preclinical data in support of such an indication will be necessary.]

It is reported that clinically eye disorders including conjunctivitis, photophobia, eye pain, keratitis and corneal lesions have been noted, some of which were transient and/or reappeared. It is further reported that patients with a plasma tyrosine concentration above 800 $\mu\text{mol/L}$, at one occasion or more, have had a significantly increased risk of developing eye symptoms compared with patients who never exceeded this plasma tyrosine level.

The eye, kidney and liver problems with NTBC are well known and it is known that high plasma tyrosine levels may cause corneal lesions. Although Nitisinone has been reported to reduce the risk of death in liver failure, prevent the occurrence of potentially fatal porphyric crisis and prevent the symptoms of tyrosinemic kidney disease, for some patients, liver transplantation may still be necessary.

Although not in a specific animal toxicity section, such problems have in general been addressed by the sponsor in the labeling. However, actual wording of these areas is under the purview of the Medical Officer.

The Warnings section of the labeling states that plasma tyrosine levels should be kept below 500 $\mu\text{mole/L}$ in order to avoid toxic effects caused by high plasma tyrosine levels, i.e. corneal lesions and hyperkeratotic lesions and that high plasma tyrosine levels may also cause neurological symptoms.

The Precautions section of the labeling states that the liver disease should be monitored regularly by liver imaging (ultra sound, CT, MR) and laboratory tests, including serum alpha-fetoprotein concentration. Occasional increase in serum alpha-fetoprotein concentration may be a sign of inadequate treatment, but patients with increasing alpha-fetoprotein or signs of nodules of the liver during treatment with ORFADIN, Nitisinone should always be evaluated for liver malignancy. It is also recommended that platelet and white cell counts be monitored regularly.

It is further recommended under the Dosage and Administration section that the treatment with Nitisinone should be initiated by a physician experienced in the treatment of hereditary tyrosinemia type 1.

Individual human sensitivity to ORFADIN, Nitisinone is not known. Although the maximal recommended dose of up to 1 mg/kg b.i.d. has been tested, apparently without unexpected or unusual incidence, doses of NTBC of 0.1 mg/kg have produced corneal damage in rats and dogs. It is concluded however, that the potential toxicity of ORFADIN is fairly well known at the proposed dose, and considering the consequence of the life threatening possibilities of the inherited metabolic defect of hereditary tyrosinemia type 1, this NDA can be approved from the standpoint of Pharmacology.

Labeling:

The Pharmacology section of the labeling for ORFADIN, Nitisinone needs to be rewritten as follows:

Carcinogenesis, Mutagenesis, Impairment of Fertility

[Nitisinone was not mutagenic in the Ames test.]

Pregnancy Category C

Adequate reproduction studies have not been conducted with _____ Nitisinone. _____

_____. Nitisinone should be given to a pregnant woman only if clearly needed.

Nursing Mothers

[]

RECOMMENDATION: AP

Pharmacology recommends approval of ORFADIN, Nitisinone for the indication of Hereditary Tyrosinemia Type 1 (HT-1). [However, should the sponsor desire an indication broader than the Orphan Drug designation for HT-1, additional and more complete preclinical data in support of such an indication will be required.]

Information to Sponsor: Labeling section above.

151
David H. Hertig
Pharmacologist

cc: Original NDA 21-232, Original IND _____ ; HFD-510 Division Files; HFD-345;
HFD-510 KDavis-Bruno, DHertig, HRhee, SYang
Recommendation: AP

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IND# →

January 26, 1995

Sponsor: Swedish Orphan AB, Stockholm, Sweden
Tel:46-08-6783380; Fax: 46-08-6783379

Agent: []

Submission Date: 12/07/1994

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW

Drug: SCO-0735: 2-(2-nitro-4-trifluoromethylbenzyl)-1,3-cyclohexanedione (NTBC)

RECOMMENDATIONS (LETTER TO THE SPONSOR)

1. Please submit the pharmacokinetic data of NTBC and its major metabolites (ED_{99} , TD_1 , $C_{ss(ave)}$, AUC_{0-24} , C_{max} , T_{max} , $T_{1/2}$, V_d) in the species used for the toxicity studies as soon as possible.

2. Re: your Study No. T-13005 (Oncogenicity Study with NTBC in Mice). Please submit the study including histopathological data, if available. In any case, the results from the dose range-finding studies and the doses selected for the rat carcinogenicity studies should be submitted for our review prior to initiation of the carcinogenicity studies.

cc: Original IND, HFD-510
A. Jordan/H. Rhee

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Herman M. Rhee, Ph. D.

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2/6

**APPEARS THIS WAY
ON ORIGINAL**

IND# _____

January 12, 1995

Sponsor: Swedish Orphan AB, Stockholm, Sweden
Tel: 46-08-6783380; Fax: 46-08-6783379

Agent: {

Submission Date: 12/07/1994

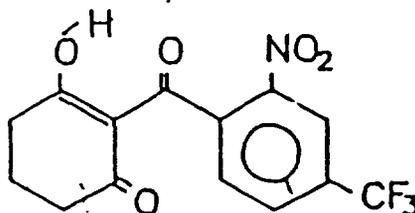
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW

Drug: SCO-0735: 2-(2-nitro-4-trifluoromethylbenzyl)-1,3-cyclohexanedione (NTBC)

Dosage form: [

Route of administration: Oral _____

Structural formula:



Indication: Hereditary tyrosinemia type 1 (Tyrosinosis, Fumarylacetoacetate hydrolyase deficiency)

Clinical: In 6 - 10 patients suffering from tyrosinemia type 1, a pharmacokinetic study will be performed after giving the drug at dose of 1 mg/kg/day for at least 4 days.

Previous human experience:

Five patients were treated with 0.1 - 0.6 mg/kg of NTBC, p.o. After 2-5 months of treatment a marked reduction in succinylacetoacetate and succinylacetone was noted in urine. The alpha-fetoprotein concentration in four patients was decreased during the treatment. Low prothobilinogen synthase

activity in erythrocytes was normalized after one week of NTBC treatment. Reduction in normalized prothrombin complex concentration, gamma-glutamyltransferase, and alkaline phosphatase in serum were also noted after 7-9 months of treatment. No adverse effects were noted as reported(Lindstedt S., et al., Lancet 340:813, 1992).

I. INTRODUCTION

Tyrosinemia I is an inherited defect of fumarylacetoacetate acetase(hydrolyase), which is the last enzyme of tyrosine degradation(Fig. 1). The disease is rare with an incidence of about 1 per 100, 000 births. The deficiency of the enzyme will lead to an accumulation of fumarylacetoacetate, succinylacetoacetone and succinylacetone, which are hepatotoxic and also potent inhibitors of porphobilinogen synthase. Acute clinical symptoms are vomiting, diarrhea, fever, edema, and hepatomegaly. Chronic symptoms are characterized by renal tubular dysfunction, an impairment of RBC metabolism, and liver failure. NTBC is supposed to alleviate some of these symptoms by an inhibition of 4-hydroxyphenylpyruvate deoxygenase(Fig. 1).

II. PHARMACOLOGY OF NTBC

A. —/R/1187: Tissue Distribution of NTBC and its Effect on Enzymes Involved in Tyrosine Catabolism in the Mouse(Report 94-05-17)

Groups of 4 male Alpk:ApfCD-1 albino mice were given ¹⁴C-NTBC at a dose of 10 mg/kg to test selective retention of the drug. Tissue radioactivity retention, tyrosine aminotransferase, 4-hydroxyphenylpyruvate deoxygenase and homogentistic acid oxidase were determined according to Lindstedt(Lancet, 1992). The drug was retained in the liver, which inhibited 4-hydroxyphenylpyruvate deoxygenase. NTBC increased the plasma level of tyrosine. No retention of radioactivity was seen in the eye.

B. —/R/1028: SC-0735 Pharmacological evaluation (Report 90-08-17)

Groups of two male albino rats were given NTBC orally at doses of 0, 50, 200 or 500 mg/kg. The animals were observed for clinical toxicity at 1, 2, 4, 6, 24 and 48 hours and on

day 6. Cardiovascular, respiratory and CNS actions of the drug were monitored by conventional methods in another group of similarly treated animals. Additionally two female guinea pigs were sacrificed to test the effects of NTBC on their isolated trachea, in vitro.

There were no pharmacologically significant effects of NTBC on heart rate, blood pressure, cardiac force, systolic time intervals or respiratory rate. There was no significant effect on tracheal muscle. In the higher dose groups of rats, NTBC prolonged the halothane-induced sleeping time.

III. TOXICOLOGY OF NTBC

A. T-12214: Stage 1 Acute Toxicity Test Battery(R-82735)

NTBC used in this study was Lot #9703x7-1. The study was preliminary so that there were no data to review.

B. T-12949: 6-week Rangefinder Probe with NTBC in Rats (— Report 88-02-17).

Ten rats/sex/group were given NTBC orally at doses of 0, 500, 2000, or 4000 ppm in diet for 6 weeks. Body weight, food consumption, general physical examinations for signs of toxicity, mortality, and corneal abnormalities were routinely monitored and clinical laboratory tests were performed.

Results:

There was no treatment-related lethality at any dose tested. NTBC produced marked decrease of BW gain gain, which was dose-dependent at all doses with no apparent sex difference. The negative effect of the drug on body weight appeared related to food consumption(Data not presented). Hyperactivity and rough/stained coat were observed in rats at the two higher dose groups. Corneal abnormalities were noted with the incidence being greater in males than females. The corneal changes include fine granularity and corneal dystrophies with and without vascularization.

Clinical laboratory changes were increases in SGOT and other hepatic enzymes. Tissue organ weight changes were also consistent with tissue wasting. Kidneys and livers from male and female rats at 500 ppm were free of significant treatment-related lesions. At the 4000 ppm dose, sciatic nerves from both sexes were mild or moderately degenerated in

all males and 8 out of 10 females, although the sponsor did not examine the control nerve.

C. T-12984: A two-year Integrated Sub-chronic/Chronic Toxicity/Oncogenicity Study in Rat

Ten CD rats/sex/group were given NTBC orally at doses of 0, 1, 5, 40, 120, 320, or 800 ppm for a year. No data were presented since this study was interrupted for some reason and 45 rats were identified to have ocular lesions in NTBC-treated groups as indicated under Study #T-13249 below.

D. T-13249: Recovery Study with NTBC in Rats — Report 88-06-27)

There were 45 rats with ocular lesions that did not recover after 13 weeks on basal diet in the study of T-12984, which was stopped after a year. The 45 rats were assigned to one of 6 groups, based on severity of their lesion: edema, mild vascularization, mild edema with moderate vascularization, severe edema, or severe vascularization.

Full ophthalmic examinations with a — slit lamp were performed at the termination of T-12984 (Prior to transfer to study T-13249), midway and at the termination.

Results and conclusion:

Recovery of ocular problems in the group 6 (severe vascularization) is summarized in Table 1. In this group as well as in the other groups, there was little or no recovery from the ocular lesions after 13 weeks on normal diet.

E. T-13255: Three Month Route of Exposure Study with NTBC in Rats (— report 89-02-20)

To determine the potential of NTBC for causing corneal lesions, a total of 151 male SD rats were given NTBC by different routes as indicated in the table below.

Group Name	NTBC Conc.	NTBC Amount	Route	Dosing Number	Experiments
10	0 ppm	0	Feed	0	Negative control
20	20 ppm		Feed		Positive control
30	1 mg/ml	1 mg/kg	p.o.	1x/day	Gavage test
40	1 mg/ml	20 µg	Ocular	2x/day	Topical
50	1 mg/ml	1 mg/kg	i.p.	1x/day	I.P. Test
60	1 mg/ml	1 mg/kg	s.c.	1x/day	S.C. Test
70	1 mg/ml	1 mg/kg	i.v.	1x/day	I.V. Test

Results:

There was no mortality. Administration of NTBC by different routes produced various types of corneal lesions (Table 2). It appears that subcutaneous route of exposure produced a greater incidence of the eye lesion in the shortest period.

F. T-12963: Six Week Dietary Rangefinding Probe with NTBC in Mice.

A 6 week dietary rangefinding study was conducted in mice. Ten mice/sex/dose were given NTBC at doses of 0, 300, 1000, 7000 or 10000 ppm for 6 weeks.

Results: Greater than 10 to 20% reduction in body weight (compared to control) were noted in male mice at 7000 and 10000 ppm doses, while females were 69 and 55% of control. Clinical signs at these doses consisted of hind limb weakness (limb dragging), rough coat, stained fur and tremors.

G. T-13005: ONCOGENICITY STUDY WITH NTBC IN MICE

NTBC was administered in diet to 50 mice/sex/group at doses of 0, 10, 350, 1500 or 3500 ppm for 6.5 months. The drug produced corneal lesions within weeks of administration, based on histopathological data. Other tissues were not examined because the drug would not be developed by —

H. T-12965 FINAL REPORT ON RANGE-FINDING STUDY IN DOGS

Two beagle dogs/sex/group were given NTBC orally at doses of 0, 1, 5, 10, 50, 100, and 150 mg/kg for 4 weeks.

Results:

a) Mortality: Treatment-related mortality was observed at dose levels of 10 mg/kg or higher (Table 3). Clinical signs were: ataxia, convulsions, diarrhea, emesis, reduced activity and stiff hindlimb gait. Symptoms that were indicative of nervous system disorder were protracted muscle activity, increased rectal temperature, poor pupillary response to light and wobbly gait.

Gross necropsy abnormalities were apparent in dogs given doses of 5 mg/kg and above and included emaciation, dehydration, evidence of gastrointestinal irritation and ocular lesions. Other toxic signs such as reduced body weight gain and gross necropsy findings suggestive of gastrointestinal irritation/hemorrhage were noted at dose levels of 5 mg/kg or above. Corneal alterations were noted

at 1 mg/kg. There was no clear difference between sexes in toxicity.

I. T-13004: 3-MONTH ORAL TOXICITY STUDY WITH NTBC IN BEAGLE DOGS

Previous study indicated that NTBC produced corneal lesions at a dose of 0.1 mg/kg in dogs. Thus, the objective of this study was to assess the reversibility of the lesions. 4 dogs/sex received NTBC orally at doses of 0, 0.1, 0.5, 1.5, or 5 mg/kg/day for 3 months.

Results:

The effect of NTBC on corneal abnormalities is summarized (Tab. 4). The positive response was confirmed in week 7 (the first scheduled examination) in 2 male and 2 female dogs. Some of the abnormalities disappeared during the recovery phase (i.e., dosing discontinuation at week 23 reduced the opacity), suggesting the reversible nature of the abnormalities.

J. T-13256: 3-MONTH OCULAR TOXICITY PROBE WITH NTBC IN RABBITS

Four male New Zealand White rabbits/group were given NTBC orally at doses of 50 or 250 mg/kg/day for 3 months.

Results:

Three rabbits out of 4 dosed at 250 mg/kg/day were found dead during the first week of the study and the fourth was terminated on the fifth day because of moribundity. Three of 4 rabbits dosed at 50 mg/kg/day were found dead prior to the end of the study (days 14, 24, and 43). The last animal was terminated on day 102. None of the animals showed signs of corneal opacity, suggesting lack of sensitivity in this species.

K. T-13587: RANGE-FINDING AND 13-WEEK OCULAR TOXICITY STUDY IN PRIMATES

To evaluate NTBC-induced ocular toxicity in primates, two male rhesus monkeys/group were given NTBC by a nasal-gastric tube at doses of 0, 0.1, 1, or 10 mg/kg/day for 2 weeks. This treatment was followed by a 2 week-recovery period. After the recovery period NTBC, 10 mg/kg, was given to 2 recovered animals for 13 weeks. The second recovery study was performed after the 13-week therapy with clinical pathology evaluation.

Results:

No general toxicity was noted, although a significant reduction in body weight was evident in an animal(#8). Ocular toxicity was negative except an animal(#8), which had a small linear corneal opacity near canthus at a dose of 10 mg/kg.

IV. TERATOLOGIC STUDY

A. T-12724: A TERATOLOGY SCREEN IN CD RATS WITH NTBC

Ten mated female CD rats/groups were given NTBC by oral gavage at doses of 0, 200, or 1000 mg/kg/day of SC-0456 from day 8-15 of gestation. SC-0456 is an NTBC related compound, of which chemical name is _____

Food consumption, body weight of dams, litter size, number of live pups, number of dead pups, gross external anomalies and total litter weight were recorded.

Results:

Maternal toxicity included reduced survival and a decrease in body weight gain with reduced food consumption at the two doses. Clinical signs of toxicity included chromorrhinorrhea, rough coat and stained head. Additional signs of toxicity included reduced activity, convulsion when handled in 2 animals and tremors after dosing in one animal. Embryotoxicity included reduced number of litters delivered and reduced pup weight with an increase in the mean percent stillborn pups/dam ratio. The mean percent pup viability from postpartum day 0 to 4 was reduced.

B. T-12725: A FERTILITY PROBE IN CD RATS WITH NTBC

Twelve sexually mature male and female CD rats were treated by oral gavage with NTBC, 100 mg/kg/day. The dosing period started 60 days prior to mating for the males and 14 days prior to mating for the females. Each male was cohabitated with one female for 7 days and the females were allowed to deliver the litters.

Results:

Paternal toxicity included salivation, chromodacryorrhea, staining of the head and thorax regions, reduced body weight, and food consumption. Eye opacities were observed in one male from the NTBC treated group.

Maternal toxicity included reduction in body weight, and food consumption. Reduction in absolute weights of the heart and

ovary was noted, which might be secondary to the reduced body weight. Reduced litter size, a decrease in percent survival of live born pups and mean pup weight were noted at birth.

V. MUTAGENICITY STUDY

A. T-12608: MUTAGENICITY EVALUATION IN S. TYPHIMURIUM

NTBC (Lot No. — 9 703-48-1) was evaluated for its ability to induce mutations in the histidine operon of Salmonella. The 4 strains tested were TA-1535, TA-1537, TA-98, and TA-100. The agents for positive control were sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene. Standard Ames assays were performed directly or in the presence of Aroclor 1254-induced rat liver (20 or 50 μ l S-9/plate) in the dose range of 0.06 to 5.0 mg/plate.

Results: Negative.

B. T-12609: MUTAGENICITY EVALUATION IN MOUSE LYMPHOMA MULTIPLE ENDPOINT TEST (FORWARD MUTATION ASSAY)

NTBC's ability to induce mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells was evaluated by standard method.

Results:

In both the direct and the activation assays there was a significant dose-related increase in mutant frequency at doses greater than or equal to 0.300 mg/ml (Tab. 5).

C. T-12610: NTBC MUTAGENICITY EVALUATION IN MOUSE LYMPHOMA MULTIPLE ENDPOINT TEST CYTOGENETIC ASSAY

NTBC's ability to induce chromosomal aberrations was evaluated in L5178Y mouse lymphoma cells over a dose range of 0.0625 to 1.000 mg/ml.

Results:

NTBC reduced relative cell growth at doses greater than 0.156 mg/ml. The inclusion of an Aroclor 1254 induced rat liver activation system did not alter the toxicity. NTBC did not reproducibly induce aberrations when tested directly. In the presence of an activation system, NTBC induced a

significant increase in sister chromatid exchanges (SCE) at all doses tested (Tab. 6). At the highest dose (0.500 mg/ml) there was a reduced number of cells that had completed two rounds of synthesis and were consequently not available for evaluation of SCE. Many of these cells contained structural aberrations which may have contributed to the division lag. It appears that NTBC was clastogenic and an inducer of sister chromatid exchange when tested in the presence of an Aroclor 1254-induced rat liver activation system.

D. T-12611: MORPHOLOGICAL TRANSFORMATION OF BALB/3T3 CELLS

BALB/3T3 cells usually cease cell division upon forming a confluent monolayer. Transformed cells on the other hand lose this contact inhibition and continue to proliferate. The colonies or foci formed by the proliferating cells show a characteristic appearance that correlates well with their ability to form tumors when injected into immunologically tolerant hosts. NTBC did not induce an increase in morphologically transformed foci when tested as a solution in DMSO at concentrations equal to or less than 0.2 mg/ml.

E. T-12664: EFFECTS OF NTBC ON HUMAN FIBROBLAST DNA

The potential ability of NTBC on DNA damage and repair induction was tested in growing human fibroblast cultures. The assays are known to be sensitive indicators of a chemical's ability to interact with and damage DNA. No significant damage was detected by either the _____ or by _____ nor were significant levels of repair induced. This study was negative at exposures of under 1 mg/ml for 1 hour.

F. T-12771: MUTAGENICITY EVALUATION IN BONE MARROW MICRONUCLEUS

a. Objective: To determine the potential genetic activity of NTBC by examining polychromatic erythrocytes of mice for the presence of micronuclei.

b. Methods: Five CD rats/sex/group were given NTBC at doses of 0, 125, 250, 500, 600, 800, 1000, and 1200 mg/kg by oral gavage. The animals were sacrificed by cervical dislocation at 24, 48, and 72 hours after the NTBC administration. The tibia and femur of both legs were used to harvest bone marrow. The marrow was suspended in 0.2 ml of newborn calf serum after centrifugation. The suspension was placed on a clean microscope slide and fixed in methanol, which was stained with 2% Giemsa in phosphate buffer. The frequency of

the polychromatic erythrocytes (PCE) in 1000 erythrocytes was determined as a measure of toxicity.

c. Results: The number of PCE/1000 erythrocytes was no statistically different from the control in both males and females. Thus, NTBC is not clastogenic.

VI. SPECIAL STUDIES

A. STUDY TO INVESTIGATE THE MORPHOLOGICAL ASPECTS OF OCULAR TOXICITY IN RATS AND THE POTENTIAL FOR RECOVERY (REPORT NO: /R/1192)

In order to characterize the nature of ocular changes-induced by NTBC, 20 male Alpk:APfSD rats/group were given the drug by gavage at doses of 0, 2, 10, or 40 mg/kg/day for 21 weeks. Since the ocular lesions developed more frequently in the 2 mg/kg group than in other groups (data not shown), half of the rats in this group were discontinued from dosing at week 14 to test for recovery from the lesions until termination at week 21.

Results:

The corneal ophthalmoscopic changes included keratitis with polymorphonuclear leukocyte and eosinophil infiltration, epithelial hyperplasia and stromal vascular in-growth. These changes were partially reversible upon NTBC withdrawal at week 14 (Table 7). By week 21 the only ophthalmoscopic change present was corneal ghost blood vessels in some animals.

B. STUDY TO INVESTIGATE THE MORPHOLOGICAL DEVELOPMENT OF THE CORNEAL LESION IN THE RAT BY LIGHT MICROSCOPY (REPORT NO: /R/1198)

Seventy male Alpk:APfSD rats were given NTBC daily by gavage at a dose of 10 mg/kg/day. Rats with corneal lesions were removed from the study at intervals so that a range of lesion, from one day old to several days old, could be investigated. After responders had been removed from the study, the remaining rats were placed on a low protein diet to establish if the dietary modification altered the progress of corneal lesion. The eyes of rats were examined by a binocular indirect ophthalmoscope after pupillary dilatation by instilling a drop of 0.5% tropicamide into the eyes. The eyes were processed for histological study in Davidson's fixative and 5 μ m thick sections were stained with hematoxylin and eosin.

Results:

Of the 70 rats on the study 48 developed corneal lesions consisting of focal areas of corneal opacity with vascularization. A low protein diet had little effect on the progress of the corneal lesions, although 5% tyrosine induces corneal lesions only in a low protein diet. Histopathological findings indicate that NTBC caused focal corneal disorganization of round basal cells. Affected cells lost the morphology of their normal counterparts so that cells at the periphery were more rounded than normal and showed increased eosinophilia. Frequently the epithelium showed separation from the underlying stroma at the point of disorganization. Slight focal polymorph infiltration in the outer stroma was noted beneath the area of epithelial disorganization. There were also inflammatory cells at the filtration angle.

C. THE EFFECTS OF L-TYROSINE SUPPLEMENTED LOW PROTEIN DIET IN THE RAT (REPORT NO: — /R/1201)

In the rat high plasma tyrosine levels have been reported to be induced by a 5% L-tyrosine supplemented low protein diet (Burns et al., Keratopathy in tyrosinemia. Birth Defects, 12, 169-180, 1976; Rich et al., Excess dietary tyrosine and corneal lesions. Exp. Eye. Res. 17, 87-97, 1973). L-tyrosine — ref. no. Y06512/001) was mixed with the diet at a 5% inclusion level. Twenty male Alpk:APfSD weanling rats were given the special diet for 7 days when cardiac blood samples were taken for plasma tyrosine analysis. The animals' eyes were routinely examined by ophthalmoscopy and histopathological evaluation was followed.

Results:

All rats fed 5% l-tyrosine showed a markedly elevated plasma tyrosine (2786 vs. 182 nmol/ml). From day 1 rats fed L-tyrosine developed multifocal areas of corneal opacity which became confluent with time. At day 3 there was fixation of the iris and by day 7 there was marked lacrimal staining of the eyelids and excessive mucus production. Histologically the eyes of all test rats showed severe keratitis and anterior uveitis. There was also severe dermatitis of the feet of test rats. The ophthalmoscopic and ocular histological changes induced by L-tyrosine in a low protein diet closely resemble those induced by the triketone chemical NTBC. The ocular toxicity-induced by NTBC is due to the raised plasma tyrosine occurring with administration of this compound rather than due to direct toxicity of the drug itself.

D. NTBC-INDUCED TYROSINEMIA IN THE DOG, RABBIT AND RHESUS MONKEY (—, R/1206)

To explore the comparative toxicology of the tyrosinemia-induced by NTBC, 6 male New Zealand white rabbits/group were given NTBC orally a single dose of 0, 10, or 50 mg/kg. Another 6 rabbits were dosed at 10 mg/kg/day for 6 weeks. Two male beagle dogs were given NTBC orally at dose of 0.5 mg/kg. Following a 90-day recovery period the dogs were given NTBC orally again at dose of 10 mg/kg. Eight male rhesus monkeys (Macca Mulatta) were given NTBC via a nasal-gastric tube at dose of 0, 0.1, 1 or 10 mg/kg/day (5 days/week) for 2 weeks, followed by a two-week recovery period. Animals were under observation for clinical signs until their sacrifice for liver enzyme preparations. Tyrosine aminotransferase was assayed in liver cytosol by the method of Schepartz (Anal. Biochem. 30: 443-448, 1969).

Results:

Rabbits given a single oral dose of 10 mg/kg had blood tyrosine levels about 2-fold higher than control in 30 min and reached almost 2000 nmol/ml in day 2. It is difficult to read from graph, but it must be was under 50 nmol/ml. Six rabbits given NTBC at 10 mg/kg for 6 weeks had blood tyrosine levels of 1500 nmol/ml. The concentration of tyrosine in the ocular fluid was 1256 ± 81 , while that of the control rabbits was about 90 nmol/ml.

Figure 2 shows the plasma level of tyrosine in two beagle dogs that were given a single oral dose of NTBC (0.5 mg/kg). The dose produced a marked and sustained tyrosinemia with a long half-life. The concentration of tyrosine in ocular fluid of each eye was 1502 and 1331 nmol/ml, while the plasma tyrosine concentration were 1842 and 1776 nmol/ml, respectively. Some of these dogs had corneal opacities.

The monkeys had no clinical signs of toxicity or ocular lesions. Plasma tyrosine levels were about 1000 nmol/ml. Eleven days after the cessation of dosing tyrosine concentrations had returned to within the normal range (figure 2). After a 4 week recovery period those animals previously dosed with NTBC were dosed again, this time all six animals received 10 mg/kg for 5 days per week for a total of 12 weeks, Plasma tyrosine measurements at weeks 4 and 7 were increased to about 1700 nmol/ml while at week 12 the values were slightly lower at 1550 nmol/ml. No changes were seen in the activities of tyrosine aminotransferase or homogentistic acid oxidase.

VII.

SUMMARY AND CONCLUSION:

Tyrosinemia type 1 is a metabolic disease, which is characterized by an elevation of hepatotoxic compounds (Figure 1) in the blood. The main site of NTBC action is to inhibit 4-hydroxyphenylpyruvate deoxygenase, which results in the prevention of tyrosine degradation. NTBC toxicity is due to an elevation of plasma levels of tyrosine, which caused ocular toxicity in rats and dogs. Rabbits and monkeys are resistant to NTBC-induced ocular opacity. Human sensitivity to NTBC is not known, although a daily dose of 1 mg/kg has been tested without unusual incidence, although NTBC (0.1 mg/kg) treatment produced corneal damage in dogs. There is minimal risk of reproduction toxicity and mutagenicity. It is concluded that the potential toxicity of NTBC is likely small at the proposed dose, considering the consequence of life threatening problems of the inherited metabolic defect.

VIII.

ATTACHMENT

7 Tables and 2 figures.

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TABLE 1
Slit Lamp Ophthalmic Exam

Group 60

Animal #		Observation First Exam		Observation 4/5/88 Exam		Observation 5/20/88 Exam
214	R	diffuse sev edema & sev vasc	R	mild edema & mod vasc	R	ghost vessels
	L	diffuse sev edema & sev vasc	L	v. mild vasc	L	ghost vessels
434	R	diffuse sev edema & sev vasc with central opacity	R	mod edema & mod vasc	R	ghost vessels
	L	diffuse sev edema & sev vasc with central opacity	L	mod edema & mod vasc	L	diffuse mod edema & mod vasc
444	R	normal	R	normal	R	normal
	L	diffuse mod edema & sev vasc	L	mild edema & mod vasc	L	ghost vessels
447	R	diffuse sev edema & sev vasc	R	v. mild vasc	R	ghost vessels
	L	diffuse mod edema & mod vasc	L	mild edema & mild vasc	L	ghost vessels
458	R	diffuse mild edema	R	diffuse mild edema	R	ghost vessels
	L	diffuse sev edema & sev vasc with corneal ulcer	L	diffuse mild edema with focal opacity	L	ghost vessels

vasc = vascularization mod = moderate sev = severe v. = very c.o. = corneal opacity

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SUMMARY OF PERTINENT EYE CLINICAL OBSERVATIONS

OBSERVATION		DOSE GROUP						
		10	20	30	40	50	60	70
CHROMODACRYORRHEA LEFT	N	0	1	0	0	2	0	0
	FIRST OBS DAY	0	56	0	0	7	0	0
CHROMODACRYORRHEA RIGHT	N	0	1	0	0	1	3	0
	FIRST OBS DAY	0	56	0	0	16	49	0
CLOUDY EYE LEFT	N	0	0	0	1	1	4	1
	FIRST OBS DAY	0	0	0	11	21	11	21
CLOUDY EYE RIGHT	N	0	3	1	0	1	2	1
	FIRST OBS DAY	0	7	41	0	56	20	7
DACRYORRHEA LEFT	N	0	1	0	0	0	0	0
	FIRST OBS DAY	0	56	0	0	0	0	0
DACRYORRHEA RIGHT	N	0	0	0	0	1	0	0
	FIRST OBS DAY	0	0	0	0	56	0	0
VASCULARIZATION EYE LEFT	N	0	0	0	0	0	0	1
	FIRST OBS DAY	0	0	0	0	0	0	7
VASCULARIZATION EYE RIGHT	N	0	0	0	0	0	1	0
	FIRST OBS DAY	0	0	0	0	0	7	0

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Table 3 Study History

Animal No.	Dose (mg/kg)	Date (1986) of		Removal reason	No. doses given	Total compound given (mg)	Ataxia	Clinical abnormal	
		Start dosing	Necropsy					Cornea	Lens
4 M	150	10/22	10/24	M.S.	2	2040	X	N.O.E.	
5 M	150	10/22	12/15	D.D.	2	1890		N.O.E.	
16 F	150	10/22	10/23	M.S.	2	1920	X	N.O.E.	
17 F	150	10/22	12/15	D.D.	2	1710	X	N.O.E.	
6 M	100	10/29	12/16	D.D.	3	2010	X	N.O.E.	
7 M	100	10/29	10/31	M.S.	2	1420		N.O.E.	
18 F	100	10/29	11/1	F.D.	3	1860	X	N.O.E.	
19 F	100	10/29	10/31	M.S.	3	2040	X	N.O.E.	
8 M	50	10/29	12/19	D.D.	9	3435	X	N.O.E.	
9 M	50	10/29	11/7	M.S.	9	3020	X	N.O.E.	
20 F	50	10/29	11/6	F.D.	8	2695		N.O.E.	
21 F	50	10/29	11/4	H.S.	6	1890		N.O.E.	
25 M	10	11/5	11/29	M.S.	21	1365	X		X
26 M	10	11/5	11/15	M.S.	10	823	X	N.O.E.	X
27 F	10	11/5	11/25	F.D.	21	1372	X		X
28 F	10	11/5	12/19	D.D.	21	1239			X
3 M	5	11/12	12/10	T.S.	28	1057.0			
10 M	5	11/12	12/10	T.S.	28	1092.0			
15 F	5	11/12	12/11	T.S.	29	900.0			X
22 F	5	11/12	12/11	T.S.	29	1130.0	X		X
11 M	1	11/12	12/10	T.S.	28	247.1			
12 M	1	11/12	12/10	T.S.	28	276.5			
23 F	1	11/12	12/11	T.S.	29	228.0			X
24 F	1	11/12	12/11	T.S.	29	266.3			

M.S.: Moribund sacrifice.
D.D.: Dosing discontinued.
F.D.: Found dead.
H.S.: Humane sacrifice.

T.S.: Terminal sacrifice.
N.O.E.: No ophthalmic exam conducted in-life.

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Table 4 Ophthalmological Examination Results

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Animal No.	Treatment (mg/kg)	Week No. ^a	Observation ^b
4M	0.1	7	Persistent pupillary membrane-L
9M	0.1	7	Stellate corneal opacity-L Linear corneal opacity-R
20M	0.5	7	Stellate corneal opacity-L
25M	1.5	7	Stellate corneal opacity-B
26M	1.5	7	Stellate corneal opacity-L, Several Linear corneal opacities-R
13F	0.1	7	Stellate corneal opacity-B
14F	0.1	7	Stellate corneal opacity-B
21F	0.5	7	Stellate corneal opacity-L
31F	1.5	7	Stellate corneal opacity-L
9M	0.1	11	Stellate corneal opacity - R
20M	0.5	11	Stellate corneal opacity-L
25M	1.5	11	Stellate corneal opacity-B
26M	1.5	11	Stellate corneal opacity-L
27M	1.5	11	Band pinpoint-corneal opacities-R
13F	0.1	11	Stellate corneal opacity-R
14F	0.0	11	Stellate corneal opacity-B
21F	1.5	11	Stellate corneal opacity-B
22F	1.5	11	Faint, circular corneal opacity-L
30F	1.5	11	Faint corneal opacity-R
31F	1.5	11	Stellate corneal opacity-L
9M	0.1	15	Stellate corneal opacity - R
20M	0.5	15	Stellate corneal opacity-L
25M	1.5	15	Stellate corneal opacity-B
26M	1.5	15	Stellate corneal opacity-L
28M	1.5	15	Faint Linear corneal opacity-L
13F	0.1	15	Stellate corneal opacity-R
14F	0.1	15	Stellate corneal opacity-B
21F	0.5	15	Stellate corneal opacity-B
31F	1.5	15	Stellate corneal opacity-L
9M	0.1	23(R)	Stellate corneal opacity-R
20M	0.5	23	Very faint wispy lines present in corneal-L
25M	1.5	23	Stellate corneal opacity-B
26M	1.5	23(R)	Stellate corneal opacity-L Several small linear corneal opacities-R

^aWeek No. followed by (R) refers to recovery (dosing discontinued).

^bAbbreviations used: R-right eye, L-left eye, B-both eyes.

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TABLE 5

VIII. SUMMARY OF MOUSE LYMPHOMA (L5178Y) RESULTS: MUTATION ASSAY : MEAN VALUE

- A. Name or Code Designation of the Test Substance: SC-0735 Technical,
— 9703-48-1), EHC-0701-1E, T-12609
B. Solvent/Vehicle: DMSO (0.01 ml/ml)
C. Test Initiation Date: 01/15/86
— Test Completion Date: 01/27/86

Test	Average Mutant Clones	Average Viable Clones	Average % Relative Growth	Average Mutant Frequency $\times 10^{-6}$
<u>NON-ACTIVATION</u>				
Medium Control	87	606	98	29
Solvent Control	83	665	100	25
0.30 μ i/ml* Test Substance	751	365	41	390
0.14 μ g/ml	85	621	71	27
0.16 μ g/ml	86	531	61	32
0.18 μ g/ml	83	497	53	33
0.20 μ g/ml	92	469	43	41
0.22 μ g/ml	85	284	22	62

: Data from one culture

Computer calculated values may vary by + 1 in the least significant digit

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TABLE 6

VIII. SUMMARY OF MOUSE LYMPHOMA (L5178Y) CYTOGENETIC RESULTS: SISTER CHROMATID EXCHANGE

- A. Name or Code Designation of the Test Substance: SC-0735 Technical (Lot No. — 9703-48-1), EHC-0701-18, T-12610
- B. Solvent/Vehicle: DMSO (0.01 ml/ml)
- C. Test Initiation Date: 08/21/85 Test Completion Date: 09/06/85
- D. Exposure times: Treatment: 4 Hours Colcecid: 2 Hours BrdU: 22 Hours

<u>TEST</u>	<u># Cells Counted</u>	<u># Chrom.</u>	<u># SCE</u>	<u>SCE/Chrom.</u>	<u># SCE/Cell</u>
<u>ACTIVATION</u>					
Medium Control	50	1951	749	0.38	15.0
	50	1935	614	0.32	12.3
Solvent Control	50	1946	695	0.36	13.9
	50	1931	730	0.38	14.6
DMS 0.025 ul/ml	50	1929	3037	1.57	60.7***
Test Substance:					
0.0625 mg/ml	50	1951	916	0.47	18.3***
0.0625 mg/ml	50	1951	991	0.51	19.8***
0.1250 mg/ml	50	1957	1049	0.54	21.0***
0.1250 mg/ml	50	1955	981	0.50	19.6***
0.2500 mg/ml	50	1960	1159	0.59	23.2***
0.2500 mg/ml	50	1930	1129	0.58	22.6***
0.5000 mg/ml	50	1924	1179	0.61	23.6***
0.5000 mg/ml	50	1972	1165	0.59	23.3***

S-9 Lot: Aroclor 1254 induced rat liver EHC-0476-19

- ** Significantly greater than solvent control $p < 0.001$ (Student's t-test)
 - ** Significantly greater than solvent control $p < 0.01$ (Student's t-test)
 - * Significantly greater than solvent control $p < 0.05$ (Student's t-test)
- Computer calculated values may vary by ± 1 in the least significant digit

Average Control Values

	<u>n</u>	<u>Act.</u>
Medium SCE/Cell	100	13.6
Medium SCE/Chrom.	3886	0.35
Solvent SCE/Cell	100	14.3
Solvent SCE/Chrom.	3877	0.37

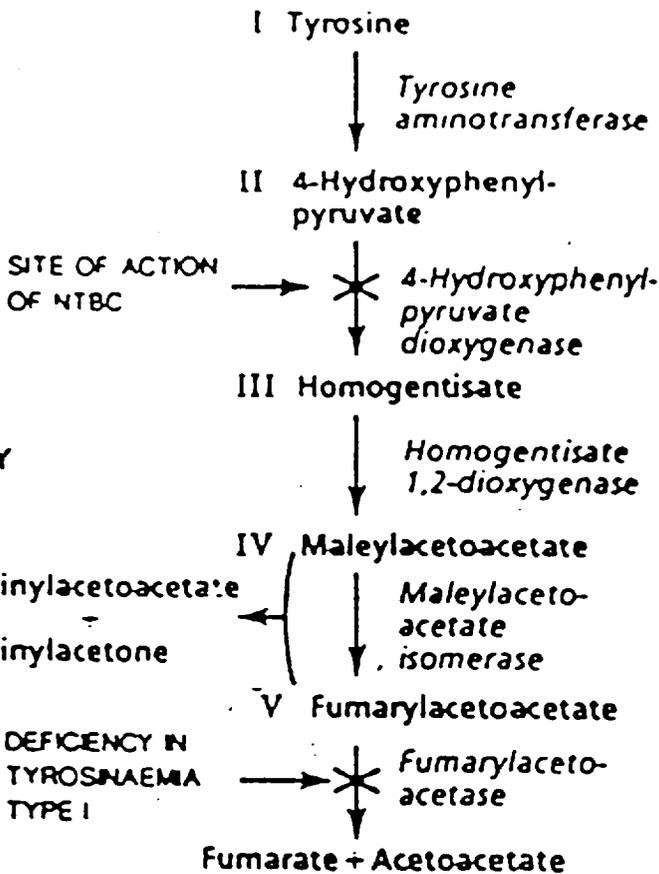
TABLE 7

HISTOPATHOLOGICAL FINDINGS IN THE CORNEA OF RATS DOSED 2mg/kg/day
SC-0735 AT WEEK 21

Histopathological Finding	Group A (continued dosing)	Group B (discontinued dosing)
No. of animals examined	8*	9
Keratitis left eye	6	0
minimal	1	0
moderate	5	0
Keratitis right eye	7	0
minimal	1	0
slight	4	0
moderate	2	0
Vascularised stroma left eye	5	4
minimal	0	1
slight	2	2
moderate	3	1
Vascularised stroma right eye	6	7
minimal	1	2
slight	1	4
moderate	4	1
Hyperplasia epithelium left eye	5	1
minimal	0	1
slight	4	0
moderate	1	0
Hyperplasia epithelium right eye	6	0
minimal	2	0
slight	2	0
moderate	2	0
Polymorph infiltration stroma left eye	6	0
minimal	1	0
slight	1	0
moderate	4	0

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TYROSINE DEGRADATION PATHWAY



PORPHYRIN SYNTHESIS PATHWAY

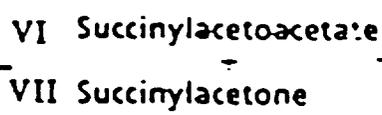
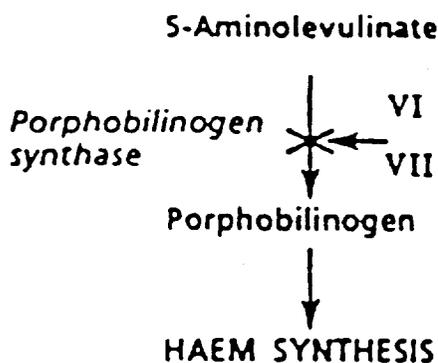
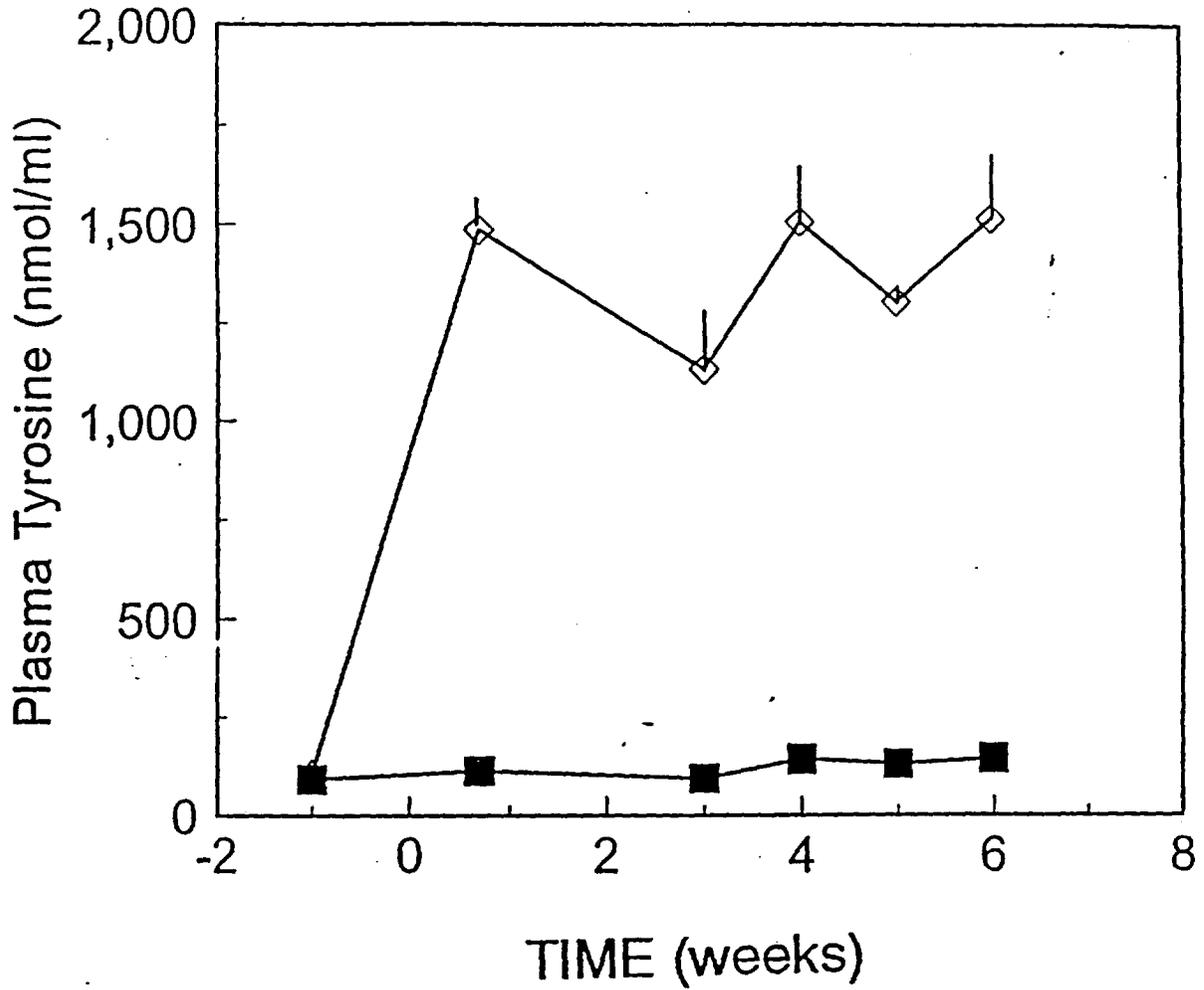


Fig 1—Tyrosine degradation and porphyrin synthesis.

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FIGURE 4
Plasma Tyrosine Profile in Rabbits Dosed with
30 μ mol SC-0735/kg/day For 6 Weeks



The rabbits were bled one week prior to dosing when the plasma tyrosine values were 104 ± 8 nmol/ml Control (■), SC-0735 treated (◇)

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