CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206709Orig1s000
207223Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 206709 (capsule) and 207223 (powder for oral suspension)
Submission date: Multiple, initial date for goal determination: December 20, 2107 for 206709 and January 19, 2018 for 207223
Drug: stiripentol
Applicant: Biocodex
Indication: seizures associated with Dravet syndrome

Reviewing Division: Division of Neurology Products

Discussion:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. Both found significant deficiencies in the nonclinical information submitted and recommended that several nonclinical studies should be conducted to address these deficiencies. The supervisor concluded that the recommended nonclinical studies could be conducted as postmarketing requirements if the drug was approved based on the clinical safety and efficacy information for this indication which may be considered an unmet medical need.

Conclusions: I agree that the nonclinical information submitted to support this NDA is deficient in the areas outlined in the primary and secondary reviews. Requesting postmarketing nonclinical studies to address these deficiencies seems appropriate.

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/s/

PAUL C BROWN
08/16/2018

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: August 16, 2018
From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 206-709 and NDA 207-223 (stiripentol, Diacomit)

NDAs 206-709 (capsule) and 207-223 (powder for oral suspension) have been submitted to support approval of stiripentol as (b) (4) treatment of (b) (4) seizures associated with Dravet syndrome. Both NDAs were submitted by the sponsor (Biocodex) as rolling submissions, with the initial submissions received on November 10, 2015, and September 5, 2017, respectively. The final set of data (abuse liability) was submitted on December 20, 2017, and January 19, 2018, respectively, and the NDAs were filed (NDA 206-709 and 207-223 Filing Communication, February 16, 2018). Communications with the sponsor were conducted under PIND 107979; no INDs were submitted.

To support the NDA, the sponsor conducted the following studies:

- Pharmacology
- PK, brain distribution, plasma protein binding
- Toxicology
    - Mouse (13-week oral toxicity)
    - Rat (21-day investigative; 26-week oral toxicity)
    - Monkey (4- and 26-week oral toxicity)
- Reproductive and Developmental Toxicology
    - Mouse (embryofetal development)
    - Rat (fertility and embryonic development, embryofetal development, pre- and postnatal development)
    - Rabbit (embryofetal development)
- Genetic Toxicology
    - Ames, in vitro chromosomal aberration, and in vivo mouse micronucleus assays
- Carcinogenicity
    - Mouse (78-week oral)
    - Rat (102-week oral)

Under PIND 107979, the sponsor was told that the lack of a 9-month oral toxicity study in nonrodent would need to be justified and that a juvenile animal study (stiripentol in combination with clobazam) would be needed but could be conducted post-approval.

The nonclinical data have been reviewed by Dr. Fisher (Pharmacology/Toxicology Review and Evaluation, NDAs 206709, 207223, J. Edward Fisher, Ph.D., July 27, 2018). Based on his review, Dr. Fisher has concluded that the nonclinical data do not support approval of the NDA, based on the following deficiencies:

- Failure of the sponsor to characterize the in vivo metabolic profile in animals or humans
- The lack of adequate plasma exposure data
- Inadequate duration of the chronic toxicity study in monkey
- Inadequate reproductive and developmental toxicity studies
- The lack of a juvenile animal toxicology study

The following is a brief discussion of the nonclinical data, focusing on the deficiencies identified by Dr. Fisher. Detailed discussion of the nonclinical data is provided in Dr. Fisher's review.

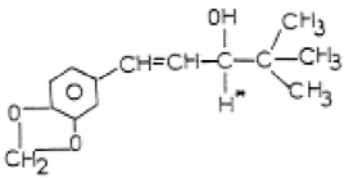
Summary and Discussion

Pharmacology

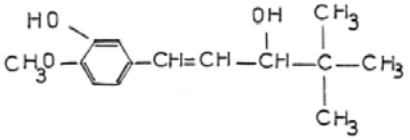
Studies by the sponsor to characterize the pharmacological activity of stiripentol were conducted primary from the early 1970s to the early 1990s and provide a limited understanding of the primary or secondary pharmacology of stiripentol. An in vitro study, conducted in 2012, assessed binding of stiripentol to only a few sites (AMPA, kainate NMDA, glycine, PCP); at none of these sites was 50% inhibition achieved at 300 µM. The anticonvulsant activity of orally administered stiripentol was assessed in the maximum electroshock (MES) and pentylenetetrazole (PTZ)-induced seizure models in mouse and rat. These animal models are typically used as initial screens for anticonvulsant potential. MES and PTZ are considered models of generalized tonic-clonic and nonconvulsive (e.g., absence, myoclonic) seizures, respectively. In the initial screening studies, stiripentol, administered orally to rodents, demonstrated anticonvulsant activity (data summarized in the table below); studies to further characterize the anticonvulsant activity were not conducted.

STUDY	SPECIES	DOSE (mg/kg)	FINDINGS	
			MES	PTZ
BC.114/GB	Swiss mouse (M)	200		20% ↓ death
		300		Delayed seizure onset; 50% ↓ death
	Wild Burgundy rabbit	100		↓ abnormal EEG
BC.098/GB	CF mouse (M)	≤2500	ED_{50} = 811.83	No protection
	SD rat (M)	n/s	ED_{50} = 410.75	ED_{50} = 373.21
BC.135/EN	OF1 mouse (M)	Stiripentol: 400-1200		Stiripentol: ↓ seizures >400 (≤90% at 1200)
		Metabolites: 200-300	D.323: no protection D.865: no protection	
	SD rat (M)	300-800		↓ seizures at all doses (60-100% at 800)
	CD1 mouse (F)	200-300	No protection	↓ seizures (10-50%)
BC.293/EN	SD rat (M)	Stiripentol: 600-1200 Enantiomers: D2232: 800-1600 D2233: 125-1400	Stiripentol ED_{50} = 735 D2232 ED_{50} = 913.5 D2233 ED_{50} = 414.9	

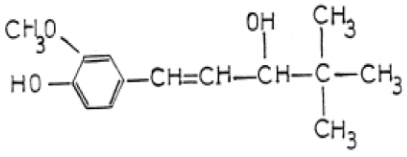
Two metabolites, stated to be "The 2 main metabolites" were tested in the MES model and had no activity. These metabolites (D.323 and D.865) were identified in a published study (Pieri F et al. Eur J Drug Metab Pharmacok 7(1):5-10, 1982; one of the authors was from Biocodex); the structures given below are from the publication.



^{3}H.STIRIPENTOL



D.323.



D.865

Stiripentol is a racemate. The two enantiomers, D2232 ((+)-STP) and D2233 ((-)-STP), were also tested in the MES model. Both exhibited anticonvulsant activity, but D2233 was more potent than D2232 or stiripentol.

Based on the data provided by the sponsor, the mechanism(s) by which stiripentol may exert anticonvulsant effects is not understood; therefore, no Established Pharmacological Class is proposed.

PK/ADME/TK

The studies conducted by the sponsor (listed below) were inadequate to characterize the PK/ADME of stiripentol.

- A study in Sprague-Dawley rat to correlate plasma stiripentol levels with anticonvulsant activity in the PTZ animal model
- A study in DC mouse and OFA rat to assess plasma protein binding
- A study to assess effects of stiripentol on metabolizing enzymes in mouse and rat (to support the carcinogenicity studies)
- A study to assess transport of stiripentol into milk of lactating goat

The only TK data provided for the pivotal toxicity studies were plasma concentrations of stiripentol; no TK parameters (including AUC) were calculated for stiripentol (even though blood samples were collected up to 24 hours post dose in some studies), the separate enantiomers, or metabolites in any animal species. The sponsor cited published studies on the pharmacokinetics of stiripentol in animals (Lin H-S, Levy RH Epilepsia 24:692-702, 1983); however, no data were provided.

According to the sponsor's Summary of Clinical Pharmacology Studies, "Limited information is available regarding circulating metabolites in [human] plasma," although stiripentol is extensively metabolized in humans following oral administration. The sponsor did provide a clinical study (BC.287) comparing the PK of stiripentol and its enantiomers; however, the clinical pharmacology team has concluded that "Without detailed analytical reports, the integrity of data obtained from this study cannot be assured" (Office of Clinical Pharmacology Integrated Review, June 19, 2018).

Toxicology
The pivotal (French GLP) oral toxicity studies were conducted in Sprague-Dawley rat and cynomolgus monkey. Stiripentol was administered for 26 weeks by oral gavage at doses of 0, 80, 220, and 800 mg/kg/day in rat and at doses of 0, 100, 250, and 600 mg/kg/day in monkey. The monkey study included a 4-week recovery period. Neither study report included a signed and dated Pathology Report.

In rat, the NOAEL was the low dose, based on renal toxicity (tubular nephrosis, basophilia, acidophilic granules accumulated in cortical tubular epithelium), centrilobular hepatocellular hypertrophy, and death (1 MDM, 2 HDF) at the mid and high doses. Toxicokinetic analyses were conducted during Weeks 4 and 26. Blood samples were collected 1, 2, 4, 6, and 24 hrs post dose at each time point; however, TK parameters were not calculated.

In monkey, a 4-week dose ranging study was conducted at oral gavage doses of 0, 100, 300, and 900 mg/kg/day in 2/sex/group. The primary findings were at the HD and consisted of renal tubular nephrosis in 1 HDM (associated with increased serum urea nitrogen) and 1 HDF. The HDF died prematurely (Day 16). In the 26-week study, the only drug-related findings were reduced rbc parameters at the high dose (males and females) and increased liver weight (with no microscopic correlates; males) at the mid and high doses. Blood samples were collected 1, 2, 4, 6, and 24 hrs post dose at each time point; however, as for rat, TK parameters were not calculated. To justify the maximum duration of dosing in monkey, the sponsor stated that target organs (kidney, liver) were similar between rodent and monkey, "no other target organs are likely to be the target organs regardless of the duration of the study," "The primate study was conducted at a time when a 6-month chronic study was the standard…", and that the results of

the completed nonclinical or clinical studies did not "suggest a study of 3 months longer in duration would provide any data that would contribute to the preclinical safety assessment."

Reproductive and Developmental Toxicology

Mouse

In CD mouse, three non-GLP embryofetal development (EFD) studies were conducted (May 28, 1974, November 29, 1974, and November 29, 1974); in all three studies, the vehicle was 0.5% gum arabic and stiripentol batch 28 was used. In the initial study, stiripentol was administered on gestation days (GD) 6-16 at oral doses of 0, 50, 200, and 800 mg/kg. The primary finding was an increase in cleft palate at the mid dose (1 fetus) and high dose (10 fetuses from 2 litters). In a follow-up study at stiripentol oral doses of 0 and 800 mg/kg (GDs 6-16), the incidence of cleft palate (2/22 vs 4/22 fetuses/litter) was increased at 800 mg/kg. In the last mouse EFD study (0, 50, 200, and 800 mg/kg on GDs 6-16) in which fetuses were examined only for skeletal and visceral, no increase in cleft palate was observed. The reason(s) for the inconsistent results was not determined. Other findings in the first two studies included increases in resorptions and decreases in fetal body weight, primarily at 800 mg/kg.

Rabbit

The non-GLP EFD study (January 15, 1975) in Wild Burgundy rabbit (stiripentol [batch 28] doses of 0 [0.5% gum arabic], 50, 200, and 800 mg/kg on GDs 8-21) was inadequate because of high rate of abortions in all groups (8, 5, 13, and 15 in C, LD, MD, and HD groups, respectively), including controls, which resulted in too few litters (13, 12, 10, and 7 litters in C, LD, MD, and HD groups, respectively) available for evaluation.

Rat

In a fertility and early embryonic development study (February 28, 1991), stiripentol was administered orally to male (71 days prior to and throughout the mating period) and female (15 days prior to mating and continuing to GD20) Sprague-Dawley rats at doses of 0, 50, 200, and 800 mg/kg. At GD 20, F_0 dams were either sacrificed for evaluation of litter and fetal parameters (12, 11, 9, and 9 in C, LD, MD, and HD groups, respectively) or allowed to deliver and rear offspring (8, 7, 10, and 10 in C, LD, MD, and HD groups, respectively). The F_1 offspring were evaluated for body weight, viability, physical and behavioral (reflex, auditory, visual) development, and reproductive function (natural delivery and rearing of offspring). The F_2 offspring evaluation was similar to that of the F_1 generation, except that auditory and reproduction functions were not assessed. The only drug-related finding was a decrease in viability of F_1 offspring during the lactation period. Because the number of dams used for assessment of embryofetal and postnatal development was substantially less than recommended, only the fertility portion of this study is adequate.

In a non-GLP pre- and postnatal development study in Sprague-Dawley rat (November 29, 1074), stiripentol was administered orally at doses of 0, 50, 200, and 800 mg/kg from GD 6 through postnatal day (PND) 23. The study was inadequate by design because of too few dams per group (10/group) and the lack of a full battery of developmental parameters. In the GLP pre- and postnatal development study in Sprague-Dawley rat (April 24, 1994), stiripentol was administered orally at the same doses (0, 50, 200, and 800 mg/kg) from GD 16 through PND 21. This study was also inadequate by design, e.g., dams were not dose throughout the period of

organogenesis and neurobehavioral development and reproductive function were not assessed in the offspring. In the GLP study, maternal toxicity was evident at the high dose (2 moribund sacrifices, adverse clinical signs, and reduced body weight gain during gestation). In the offspring, decreased survival (PND 1-4), reduced body weight gain, and adverse effects on reflex (air and surface righting) development were observed at the high dose; physical development appeared unaffected.

Genetic Toxicology
The sponsor conducted a standard battery of genotoxicity assays. All assays were conducted under French or U.S. GLP, except for the Ames assay. Dr. Fisher found the assays to be adequately conducted and negative for mutagenicity and clastogenicity, except for an in vitro chromosomal aberration assay in CHO cells. However, as he notes, positive responses (±S9) in that assay were observed only at a concentration associated with excessive cytotoxicity. Therefore, overall, stiripentol is considered to not have genotoxic potential.

Carcinogenicity
The carcinogenic potential of stiripentol was assessed in a 78-week oral (gavage) study in CD-1 mouse (0, 0, 60, 200, and 600 mg/kg/day) and a 102-week oral (gavage) study in Sprague-Dawley rat (0, 0, 80, 220, and 800 mg/kg/day). Both studies were conducted under French GLP; however, no signed and dated Pathology Report was provided for either study. Dose selection for the study in rat was based on a 13-week study that was not submitted to the NDA. In the mouse study, there was an increase hepatocellular (adenoma, carcinoma, combined adenoma/carcinoma) tumors in males and females at the mid and high doses. The study in rat was negative for drug-induced tumors.

Based on the short duration of the mouse study and the lack of a separate Pathology Report for either study, the assessment of the carcinogenic potential of stiripentol is considered minimally acceptable.

Conclusions and Recommendations
The nonclinical studies of stiripentol conducted by the sponsor are inadequate to support approval of the NDA, as discussed above. Deficiencies include the lack of a chronic toxicity study in nonrodent of sufficient duration, an inadequate battery of reproductive and developmental toxicology studies, and a lack of characterization of the PK/ADME/TK of stiripentol. However, because of the severity of the indication and the age of the intended patient population, if the clinical team concludes that stiripentol is efficacious and addresses an unmet need and that the human safety data are adequate to support approval, the deficiencies in the nonclinical data may be addressed postmarketing. (A toxicity study in nonrodent of 9 months' duration and bridging TK data would not be needed if the clinical safety data are considered adequate.)

The following postmarketing requirements (PMR) are recommended:

- Embryofetal development studies in two species (rodent and nonrodent)
- Pre- and postnatal development study in rodent
- Juvenile animal toxicology study in one species

- Studies to characterize the in vivo metabolic profile of stiripentol in the species and strain of animals used in the pivotal reproductive and developmental studies and carcinogenicity studies.
- Toxicokinetic bridging studies in the species and strain of animals used in the pivotal reproductive and developmental and carcinogenicity studies.

If the quantitative evaluation of metabolites in humans (to be conducted post-approval if the NDA is approved) documents the lack of any major circulating metabolites (i.e., one accounting for >10% of total drug-related material), then studies to characterize the in vivo metabolic profile in animals would not be needed. If major circulating metabolites are identified in humans, selection of species for the PMR developmental studies should take into consideration interspecies differences in the in vivo metabolic profile. Based on the results of the PMR studies, it is possible that additional nonclinical studies may be needed.

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/s/

LOIS M FREED
08/16/2018

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER(S):	206709, 207223
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/20/17
PRODUCT:	Diacomit (stiripentol) Capsules (206709) Powder for oral suspension (b) (4) (207223)
INDICATION:	Seizures associated with Dravet syndrome
SPONSOR:	Biocodex
REVIEW DIVISION:	Division of Neurology Products (DNP)
PHARM/TOX REVIEWER:	Ed Fisher
PHARM/TOX SUPERVISOR:	Lois Freed
DIVISION DIRECTOR:	Billy Dunn
PROJECT MANAGER:	LaShawn Dianat

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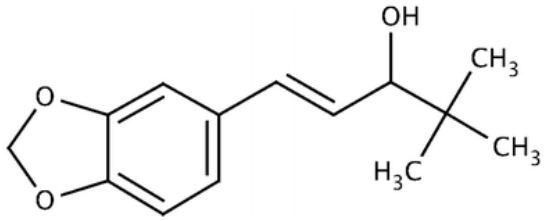
Note: All figures and tables in this review were excerpted from the sponsor's submission or literature unless otherwise noted.

I. EXECUTIVE SUMMARY

A. Drug

Trade name:	Diacomit
Generic name:	Stiripentol
Code name:	D 306
Chemical name:	$C_{14}H_{18}O_3$
CAS registry number:	49 763-96-4

Structure:



Drug class:	Anticonvulsant
Indication:	Seizures associated with Dravet syndrome
Clinical dose:	50 mg/kg/day, administered in 2 or 3 divided doses
Dosage forms:	Capsules, Powder for oral suspension
Relevant IND:	107979

B. Background and brief discussion of nonclinical findings

Stiripentol (STP) is a pentenol derivative that is structurally different from other known antiepileptic drugs (AEDs). The mechanism of anticonvulsant action remains unclear. Several possible mechanisms of action have been proposed, including direct effects mediated through the gamma-aminobutyric acid $(GABA)_A$ receptor and indirect effects involving inhibition of cytochrome P450 activity (demonstrated for CYP1A2, CYP2C9, CYP2C19, and CYP3A4). STP was shown to inhibit the oxidative metabolism of other drugs when co-administered, resulting in increased plasma concentrations of AEDs such as carbamazepine (CBZ) and phenytoin that undergo metabolism by cytochrome P450 enzymes. STP inhibited the formation of the reactive epoxy metabolite of CBZ, which was thought to provide an additional benefit, since toxicity, including teratogenicity, has been attributed to the epoxide (Finnell et al., 1995). A similar metabolic interaction was seen with clobazam (CLB): STP inhibited the N-demethylation of CLB to the active metabolite N-desmethylclobazam (NCLB) and more strongly inhibited the hydroxylation of NCLB to the inactive 4'-hydroxy-N-desmethylclobazam (Tran et al., 2005). This metabolic interaction could improve the effectiveness of CLB by increasing plasma levels of CLB and NCLB in patients treated with the CLB/STP combination. However, STP also demonstrated independent anticonvulsant activity in several animal models, including the maximal electroshock (MES) and pentylenetetrazol (PTZ) models, with what were considered (b) (4) to be adequate

protective indices (TD50/ED50). In other studies providing evidence for intrinsic activity, STP increased GABAergic postsynaptic currents when applied to hippocampal CA3 pyramidal neurons in immature rats at clinically relevant concentrations (therapeutic doses of STP are reportedly associated with plasma concentrations of 4 to 22 µg/mL), was effective in a rodent model of refractory pilocarpine-induced status epilepticus, attenuated seizure activity in Nav1.1 mutant zebrafish, which spontaneously exhibits abnormal electrographic activity and convulsive behavior, and was effective in preventing hyperthermia-induced seizures in young (1 month of age) but not older (5 months of age) animals in a heterozygous SCN1A mutant mouse model of Dravet syndrome that presents with hyperthermia-induced seizures. It should be pointed out that very few of these pharmacology studies of STP were carried out by the sponsor (only very early anticonvulsant testing in basic models, including one conducted (b) (4) for the sponsor, and pharmacologic interaction studies), so most of this information comes from literature reports.

Clinical evidence cited by the sponsor to support direct anticonvulsant activity included the results of an observational study performed in Japan on 23 patients with Dravet syndrome, in which 48-61% of patients experienced a more than 50% decrease in seizure frequency, rates that are close to those observed in studies conducted in Europe, although only 1/2 of the Japanese patients received CLB as co-medication, and 20% of them had a CYP2C19 inactive allele *2 or *3. Post hoc analysis of the pharmacokinetic data from two randomized controlled trials performed in patients with Dravet syndrome in France indicated that responder rate was similar whether the patients exhibited an increase in CLB, NCLB, or NCLB/CLB on STP or not. For example, 78% of children experienced an increase in the NCLB/CLB ratio, but their responder rate (65%) was not different from those who did not (75%). These data are thought by the sponsor to show that the anticonvulsant effect of STP in Dravet syndrome does not depend only on its pharmacokinetic interaction with CLB (Chiron, Orphan Drugs: Research and Reviews 2014:4 29–38).

Mechanism of action studies have focused on GABA potentiation. Literature studies indicate that STP is a positive allosteric modulator of the GABAA receptor at a site distinct from that of other GABAA receptor modulators. In studies conducted by the sponsor, co-administration of STP and a benzodiazepine (diazepam or clonazepam) was reported to result in more than additive protection against metrazol-induced seizures in mice. Although the data were not convincing in showing more than additive effects, these results appeared to be consistent with in vitro studies (reported in literature) showing that when STP at clinically relevant concentrations was applied to brain slices together with diazepam, a further enhancement of GABAergic neurotransmission was observed. The additive effects of STP and BZDs suggested that the combination would produce stronger enhancement of GABAergic inhibition and potentiate a greater variety of GABAA receptors than would BZDs alone. In addition to possible allosteric modulation of the GABAA receptor, STP was recently reported (in literature) to increase GABA release through a presynaptic effect.

Formal safety pharmacology studies were not conducted. In a CV study conducted in 2 mongrel dogs, STP decreased blood pressure and heart rate slightly at both doses tested (2.5 and 5 mg/kg iv). In the (b) (4) study, high doses of STP (600 to 1800 mg/kg ip) produced neurological deficits (inability to remain on the rotarod, decreased motor activity) and decreased respiration in mice. At the highest dose, decreased motor activity, spasticity, ataxia, reduced rotarod performance, sedation, ptosis, muscle relaxation, loss of righting reflex, and decreased respiration with cyanosis were observed and 1 of 2 animals died.

ADME information was based on literature reports. Based on studies in rat and monkey, STP formulated in 1% carboxymethyl cellulose (CMC) or PEG 400 appeared to be rapidly absorbed after oral administration, with peak levels seen at ~1 hour. In rhesus monkey (not used in toxicity testing), the extent of oral absorption was <30%, which was attributed to poor solubility and a hepatic first pass effect. Distribution studies in rats indicated that labeled STP partitions into liver, adrenal gland, kidney, lung, placenta, brain, spinal cord, and fetus. Brain/plasma ratios in rats varied between 0.25 and 0.70 with no difference between (R)-(+) and (S)-(-) enantiomers. Chiral

inversion of (R)- to (S)-STP was found following oral administration in the rat. When racemic drug was administered, (S)-STP was predominant in rat plasma, with an AUC S/R ratio of 3.3. In humans, (R)-STP was the predominant enantiomer after oral administration of the racemate (1200 or 2400 mg), yielding an AUC R/S ratio of 4.1. Enantioselectivity was observed with respect to anticonvulsant activity: (R)-STP was 2.4 times more potent than its antipode against PTZ-induced clonic convulsions in rats.

Based on literature studies in which urinary metabolites were characterized in Sprague-Dawley (S-D) rats and rhesus monkeys, the metabolism of STP is thought to primarily involve glucuronidation, opening of the methylenedioxyphenyl ring, and hydroxylation. Thirteen of 15 rat urinary metabolites were found in common with human urinary metabolites. STP was also extensively metabolized in rhesus monkey and the urinary metabolite profile agreed qualitatively with the findings in rats and humans. Excretion studies showed that in rat and monkey the fraction of dose appearing unchanged in urine is ≤1%, consistent with elimination by metabolism. A multiphase elimination curve was seen in rats after iv administration of ^{3}H-STP, with plasma concentrations dropping rapidly in the first phase, followed by a much slower decrease during the second phase. The same multiphasic pattern of STP disappearance from plasma was observed in monkeys following iv administration. Plasma clearance varied with dose in this study, indicating nonlinearity, and Michaelis-Menten type nonlinear pharmacokinetics has been demonstrated for STP in humans.

The toxicity of STP was evaluated following repeated oral (gavage) administration to mice (13 weeks), rats (6 months), and monkeys (1 and 6 months). While the chronic toxicity and carcinogenicity studies were GLP compliant, some of the deficiencies, judged by current standards, include limited TK data (exposures in terms of AUC not calculated) and a chronic nonrodent (monkey) toxicity study that was only 6 months in duration. In addition, the Ames test and embryofetal development studies were not conducted according to GLP, and an adequate pre- and postnatal development study with dosing throughout gestation (from implantation) and neurobehavioral testing was not conducted. And, because only limited comparative metabolism data were provided, the human relevance of the animal models has not been established. Notable toxicity findings included renal toxicity in rats and monkeys, hepatic tumors in mice, and teratogenicity in mice. Plasma STP levels (Cmax) associated with the no-effect doses for renal histopathology and liver tumors were similar to those expected clinically. Plasma levels were not determined in the embryofetal studies, but the no-effect dose for increased malformation incidences in mice was below the human dose on a body surface area (mg/m^2) basis.

In the 13-week (CD-1) mouse study, oral (gavage) administration of STP (0, 60, or 800 mg/kg/day) resulted in deaths that were considered possibly drug-related and adaptive liver changes (increases in cholesterol, liver weight, and hepatic cell hypertrophy) at the HD (Cmax ~ 60 ug/mL). Peak plasma STP concentrations at the no-effect dose (LD) were approximately 15 ug/mL in both sexes.

In the 26-week (S-D) rat study, oral (gavage) administration of STP (0, 80, 220, or 800 mg/kg/day) resulted in deaths, clinical signs, decreased body weight (BW), and clinical chemistry, urinalysis and macroscopic and microscopic evidence of liver and kidney toxicity at the MD and HD. The liver changes (hepatocellular hypertrophy) appear to have been adaptive, but the renal histopathology (increased incidences of tubular nephrosis, tubular basophilia, accumulation of acidophilic globules in the cortical tubular epithelium) associated with increases in plasma urea and electrolytes and proteinuria was clearly adverse. Reversibility was not assessed in this (or any) study. At the no-effect dose, peak plasma levels were approximately 4 and 11 ug/mL in males and females, respectively.

Renal toxicity was also reported in the 4-week (cynomolgus) monkey study, in which oral (gavage) administration of STP (0, 100, 300, or 900 mg/kg/day) resulted in death (1 female), clinical signs (hypotonia, sedation), decreased BW gain, increased blood urea level, increased

liver weight, and renal tubular nephrosis at the HD. The no-effect dose (300 mg/kg) was associated with peak plasma STP levels of approximately 24 ug/mL in both sexes.

In the 6-month (cynomolgus) monkey study, oral (gavage) administration of STP (0, 100, 250, or 600 mg/kg/day) resulted in dose-related decreases in RBC parameters at all doses and increased liver weights and possible renal toxicity (tubulo-interstitial nephropathy) at the MD and HD. While the renal effects were not as pronounced in this study, maximal plasma levels of STP at the HD were only about 20 and 24 ug/mL in males and females (mean values), respectively. Effects on RBC parameters have also been seen clinically (see clinical review by Steven Dinsmore).

In in vitro genotoxicity assays, STP was negative in a (non-GLP) Ames test, HPRT gene mutation assay in V79 Chinese hamster cells, UDS assay using mice and rat hepatocytes, and a chromosomal aberration assay in human lymphocytes. In the chromosomal aberration assay in CHO cells, STP concentrations that were considered cytotoxic based on morphological changes (60 and 150 ug/mL) increased incidences of aberrant cells in both the presence and absence of S9. STP was negative in the in vivo mouse micronucleus test. (All assays except the Ames test were GLP.)

Carcinogenicity studies conducted in mice and rats appeared adequate (see CDER Exec-CAC minutes dated 7/26/18). In CD-1 mice, oral (gavage) administration of STP (0, 0, 60, 200, or 600 mg/kg/day) for 78 weeks increased the incidences of liver adenomas and carcinomas at the MD and HD. This was not an unexpected result for a demonstrated hepatic enzyme inducer in mice. The no-effect dose for tumorigenic effects in the mouse study (60 mg/kg/day) was associated with peak plasma STP levels of ~20 µg/mL. In S-D rats, oral (gavage) administration of STP (0, 0, 80, 220, or 800 mg/kg/day) for 102 weeks resulted in BW reductions and non-neoplastic adaptive liver changes (hepatocellular hypertrophy and intracytoplasmic concentric inclusions) at the MD and HD but did not produce any clear evidence of carcinogenicity. The renal histopathological finding (tubular nephrosis) prominently observed in the 6-month general toxicity study in rats was not increased in males in this study, presumably due to increased background incidences. However, dose-dependent increases in chronic interstitial tubular nephrosis were seen in females and contracted glomerular tuft was increased in treatment groups of both sexes, as seen in the 6-month study. The highest dose tested in the rat study was associated with peak plasma STP levels of ~30-35 µg/mL.

In a GLP study that was intended to address reproductive performance and pre- and postnatal development, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to male and female S-D rats prior to and during mating and throughout pregnancy and lactation in females did not result in any clear effects on fertility or reproductive performance. The HD induced some maternal/paternal toxicity (clinical signs in females, mortality in 1 male and 2 females, reduced BW gain of males and females). The only developmental toxicity noted was decreased viability of pups during lactation at the HD. No signs of toxicity and no effects on fertility were noted in F1 animals and the development of F2 pups was not affected. This study can be considered an adequate reproductive toxicity assessment, but the numbers of litters examined at term for abnormalities and evaluated postnatally were too small for this to be considered an adequate developmental toxicity (embryofetal or pre- and postnatal) study. In addition, no neurobehavioral evaluations of offspring were conducted.

Three non-GLP mouse embryofetal development (EFD) studies were conducted. In the initial study, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg) to pregnant CD mice throughout organogenesis resulted in increased resorptions and decreased fetal BWs at all doses and an increased incidence of malformations at the HD, with no evidence of maternal toxicity. Cleft palate was seen in 1 MD fetus and 10 fetuses from 2 HD litters. In a follow-up study conducted with only C and HD groups (0 or 800 mg/kg by oral gavage) and an expanded dosing period (GDs 4-14), the results again indicated developmental toxicity (increased mortality, decreased BW, increased malformation incidence) in the absence of maternal toxicity at the

single dose tested. Incidences of external malformations (only external examinations were conducted) were increased in the drug-treated group: the number of litters with malformed fetuses (8/22 vs 4/22) and with fetuses with cleft palate (4/22 vs 2/22) was doubled in the drug-treated group compared to C. However, when a third study was conducted in which STP (0, 50, 200, or 800 mg/kg) was given orally (by gavage) to pregnant CD mice throughout organogenesis (GDs 6-16) and only skeletal and visceral fetal examinations were performed, there no effects on incidences of abnormalities.

In a non-GLP oral (gavage) rabbit (wild burgundy strain) EFD study of STP (0, 50, 200, or 800 mg/kg/day), drug-related developmental (increased resorption at MD and HD, decreased fetal BW at all doses) and maternal (decreased BW gain and increased abortion at the MD and HD) toxicity was observed, but there were no apparent drug-related increases in incidences of malformations or variations.

Two studies examining some aspects of pre- and postnatal development were submitted, but neither can be considered adequate. In the first (non-GLP) study, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to female S-D rats from GD 6 through PND 23 resulted in maternal (decreased BW gain at the HD) and developmental toxicity (decreased pup BW at birth and increased mortality during lactation at HD, decreased BW gain during lactation at MD and HD). While the dosing period was adequate, the number of litters per group (10) and the postnatal assessments conducted were not. No neurobehavioral evaluations of offspring were conducted. In the second (GLP) study, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to female S-D rats from GD 16 through PND 21 resulted in maternal (mortality, clinical signs, decreased BW gain at the HD) and developmental toxicity (decreased pup survival during PNDs 1-4, decreased pup BW at birth and throughout lactation, deficits in reflex development at HD). While this study used adequate N's (20/grp), the dosing period (starting on GD 16) and the offspring assessments (no reproductive or neurobehavioral evaluations) were inadequate.

A critical deficiency in the nonclinical safety assessment of STP for the intended pediatric indication is the lack of any juvenile animal toxicity studies.

C. Recommendations

The application is not approvable from a pharmacology/toxicology standpoint due to the failure to adequately characterize metabolism across species, the lack of adequate drug exposure data, the inadequate duration of the chronic non-rodent toxicity study, and an inadequate nonclinical safety assessment of the potential for STP to induce developmental toxicity during embryofetal, pre- and postnatal, and juvenile exposure.

II. PHARMACOLOGY

A. Brief summary

The anticonvulsant profile of STP (61081) was studied in mice and rats by the [redacted] (b) (4) [redacted] (b) (4) Biocodex Study BC.098/GB, 1982). In the standard models, the activity of STP was compared to that of the prototype AEDs, phenytoin, phenobarbital, ethosuximide, and valproate (Table II.A.1). The report concluded that the profile of anticonvulsant activity of STP differed significantly from and did not compare favorably with those of the prototype agents. Plasma levels were not determined in this early study.

Table II.A.1. Anticonvulsant profile of oral STP and prototype AEDs in mice and rats

ADD No. Substance	Time of Test (hrs)		TD50 (mg/kg)		MES-ED50 (mg/kg)		sc Met-ED50 (mg/kg)	
	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats
61081	-,4,4	-,4,4	No rotorod toxicity up to 2500	No ataxia up to 2000	811.83 [3.10] * (744.78-984.25) [7.49]	410.75 [>4.87] * (370.45-461.57) [15.77]	* No protection up to 2500	373.21 [>5.36] * (250.96-515.96) [3.46]
Phenytoin	2,2,2	-,4,4	86.71 (80.39-96.09) [13.01]	No ataxia up to 3000	9.04 [9.59] (7.39-10.62) [6.28]	29.82 [>100] (21.92-38.91) [2.82]	[<0.29] No Protection up to 300	[ ] No Protection up to 800
Pheno-barbital	2,2,2	½,5,5	96.78 (79.88-115.00) [8.51]	61.09 (43.72-95.85) [3.00]	20.09 [4.82] (14.78-31.58) [5.20]	9.14 [6.68] (7.58-11.86) [4.12]	12.59 [7.62] (7.99-19.07) [3.84]	11.55 [5.29] (7.74-15.00) [4.08]
Etho-suximide	1,½,½	2,2,2	879.21 (839.89-933.51) [30.50]	1012.31 (901.66-1109.31) [15.33]	[<0.44] No protection up to 2000	[<0.84] No protection up to 1200	192.21 [4.56] (158.59-218.44) [7.39]	53.97 [18.76] (45.57-60.85) [9.05]
Valproate	2,1,1	1,½,½	1264.39 (800-2250) [4.80]	280.26 (191.32-352.76) [4.63]	664.80 [1.90] (605.33-718.00) [18.17]	489.54 [0.57] (351.14-728.37) [2.90]	388.31 [3.26] (348.87-438.61) [8.12]	179.62 [1.56] (146.73-210.35) [8.62]

( ) 95% Confidence interval
[ ] Slope, regression line
* Protective Index (P.I.)=TD50/ED50

In other studies reported in the literature, STP increased GABAergic postsynaptic currents when applied to CA3 pyramidal neurons of hippocampus in immature rats at what were considered clinically relevant concentrations (therapeutic doses of STP reportedly result in plasma concentrations of 4 to 22 µg/mL), was effective in a rodent model of refractory pilocarpine-induced status epilepticus, attenuated mutant seizure activity in Nav1.1 mutant zebrafish that spontaneously exhibits abnormal electrographic activity and convulsive behavior, and in preventing hyperthermia-induced seizures in young (1 month of age) but not older (5 months of age) animals in a heterozygous SCN1A mutant mouse model of Dravet syndrome that presents with hyperthermia-induced seizures.

A number of mechanism of action studies reported in the literature were summarized in the NDA. In hippocampal slices, STP (30-300 µM) concentration-dependently increased duration and frequency of miniature inhibitory GABAA receptor-mediated currents (mIPSCs) without modifying amplitude or rise time. This effect was observed in CA3 pyramidal cells as well as in CA1 pyramidal cells and granular cells. The effect could not be ascribed to an interaction with GABA transporters, benzodiazepine, or neurosteroid binding sites. STP did not to bind to the GABAA, GABAB, strychnine, or benzodiazepine receptors at concentrations up to 10^{-4} M. Patch-clamp recordings from transiently transfected mammalian cells showed that STP acts as a positive

allosteric modulator of the GABAA receptor; STP (0.1-100 μM) concentration-dependently potentiated the response to GABA alone. In all these experiments, STP was shown to shift the GABA concentration response curve to the left, but did not increase maximal response, indicating allosteric modulation of GABAA receptors. STP (100 μM) also increased the decay time of evoked IPSCs in dentate granule cells that are mediated by the GABA-A receptor. The co-application of STP (100 μM) and CLB (10 μM) significantly increased IPSC decay time (351±38% of control; $p<0.001$). This increase was greater than that induced by CLB or STP alone, suggesting that these drugs act at separate loci. This conclusion is further supported by the observation that flumazenil blocked IPSC prolongation induced by diazepam (1 μM) and CLB (10 μM) but had no effect on IPSC prolongation induced by STP. In addition to acting postsynaptically at a site associated with the GABA-A receptor, STP may act presynaptically to cause an increase in GABA release. STP (100 μM), but not diazepam (1 μM), significantly increased spontaneous miniature excitatory post-synaptic currents (mEPSCs) in the dentate gyrus by increasing glutamate release from nerve terminals. However, following prolonged seizures, STP was no longer able to increase glutamate release.

In a literature study of the concentration/response relationship for elevation of the PTZ threshold dose for induction of clonic convulsions in rats after ip or po administration of STP (Shen et al., 1992), significant elevation in PTZ threshold dose was observed at a single ip dose of 300 mg/kg or at STP plasma levels exceeding 35 ug/mL. The maximal anticonvulsant response (dose ratio of 3) was reached with doses at or above 450 mg/kg (or plasma concentration ≥ 120 ug/mL), accompanied by the appearance of neurotoxicity. Subchronic treatment consisted of 9 consecutive oral doses of STP (150, 400, or 800 mg/kg) over a 3-day period, until steady-state plasma concentrations were attained (respective steady-state levels of 33, 61, and 116 ug/mL). The maximal anticonvulsant effect (ratio of the post-drug to the baseline threshold dose) was not reached even at the highest dose of 800 mg/kg (Figure II.A.1). The mean plasma concentration of STP at steady state was 10 ug/mL in the French randomized controlled trial, with a mean STP dose of 49 mg/kg/day (Chiron, 2014).

Figure II.A.1

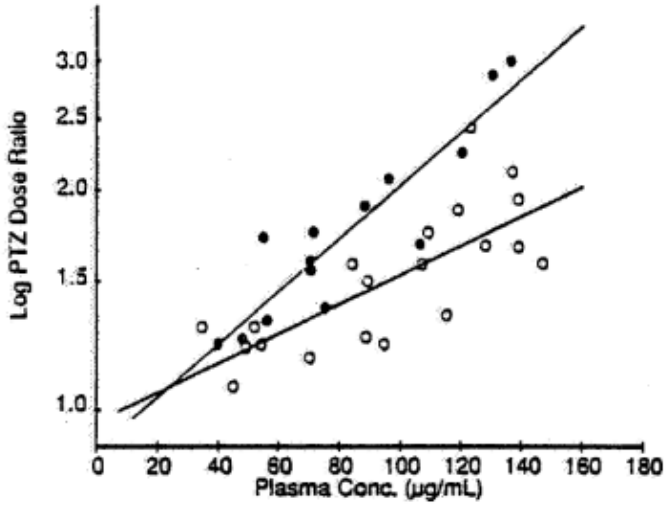


Fig. 1. Relationship of anticonvulsant effect, expressed as $\log_{10}$ PTZ threshold dose ratio, to plasma drug concentration after acute intraperitoneal (●) and subacute oral (○) treatment with stiripentol.

B. Safety Pharmacology

No formal safety pharmacology studies were conducted by the sponsor. However, in an early CV evaluation conducted in 2 mongrel dogs (Study BC.114/GB, conducted in 1974), STP (2.5 and 5 mg/kg iv) decreased blood pressure (up to 20%) and heart rate at both doses tested. This study reported no effects respiration rate or amplitude in the same dogs at these doses. During the [redacted] (b) (4) study, the effects of high doses of STP (600 to 1800 mg/kg ip in groups of 2 animals) were measured in male CF1 mice. At the 600 mg/kg dose, neurological deficits (ataxia and decreased motor activity) and decreased respiration with cyanosis were observed; animals recovered within 1 hour. The same symptoms were observed at the 1200 mg/kg dose, but they lasted longer (2 hours). At the 1800 mg/kg dose, decreased motor activity, spasticity, "ataxia," reduced rotarod performance, sedation, ptosis, muscle relaxation, loss of righting reflex, and decreased respiration with cyanosis were observed and 1 of 2 animals died.

III. PHARMACOKINETICS

A. Absorption

The only study conducted by the sponsor that addressed absorption was a study examining the relationship between plasma STP levels and anticonvulsant effects against sc PTZ-induced convulsions in S-D rats. In that study, peak STP levels were reached between 4 and 8 hours after oral (gavage) administration of 300, 600, or 800 mg/kg (suspended in 30% PEG 400) and the anticonvulsant effect generally correlated with plasma level. Complete protection was associated with a plasma concentration of 71 ug/mL. Plasma levels and anticonvulsant activity were maintained for up to 24 hr at the highest dose.

In a study reported in the literature (Lin and Levy, 1983), the absolute bioavailability of STP (80 mg [~20 mg/kg] dissolved in PEG 400) after oral (gavage) administration to rhesus monkeys was 0.21. The low bioavailability was attributed at least partially to an hepatic first-pass effect. Based on the STP clearance measured after iv administration, a blood/plasma ratio of 0.6 and a hepatic blood flow value of 2.74 L/h/kg, a maximum bioavailability of 0.33 was predicted assuming elimination by hepatic metabolism.

B. Distribution

The only study conducted by the sponsor was a plasma protein binding study. In that study, plasma kinetics of STP and its metabolites were measured after oral (gavage) administration of ^{3}H-STP (200 mg/kg in 1% CMC) to mice and rats. In mice, the maximum concentration of STP was seen at ~6 hr and was higher in females than males. The maximum concentration observed in mice was greater than that in rats, for both sexes. In rats, maximum concentrations seen at ~15 hours were similar between males and females. Plasma protein binding was high in both species. In mice, maximum binding appeared after 15 minutes in males (96%) and was around 90% after 12 hours. In females, protein binding was 94% after 15 minutes and maximal after 12 hours (95.8%). In rats, maximal protein binding was 94% between 6 to 15 hours, and there did not appear to be any difference between the sexes.

In a study reported in the literature, following iv administration of ^{3}H-STP to Wistar rats, the concentration-time curve indicated a two-phase elimination, with a rapid phase (t1/2 = 1 hr) followed by a slower phase lasting more than 24 hr (t1/2 = 13 hr). Non-linearity was demonstrated after iv administration to rhesus monkeys, with dose-dependent clearance. Vd was large (1 L/kg), indicating a high degree of tissue distribution and was not dose-dependent. Distribution studies indicated that STP partitions extensively in various tissues (liver, adrenal gland, kidney, lung, placenta, brain, spinal cord and fetus).

Preliminary results of a PK study in 35 children with Dravet syndrome (median age 7 years, range 1–17 years) receiving STP (median dose 45 mg/kg/day, range 27–89 years) in addition to valproate and CLB showed that apparent Vd, apparent oral clearance, and elimination t1/2 of STP increased as body weight increased from 10 to 60 kg: from 32 to 192 L, 2.6 to 5.7 L/hour, and 8.5 to 23.5 L/hours, respectively (Chiron, 2014).

C. Metabolism

The only metabolism study of STP conducted by the sponsor was a study of enzyme induction in mice (CD-1) and rats (S-D). In that study, enzymatic activity in liver microsomes was examined after oral (gavage) administration of STP (60, 200, or 600 mg/kg in mice; 80, 220, or 800 mg/kg in rats). The results indicated increased EROD (5 and 25X in mice and rats), phenacetin deethylase (3-6X), and PEROD (4-7X) activities in both species, increased glucuronyl transferase activity in rats (both sexes) and male mice, and decreased dextromethorphan demethylase activity at the highest dose in both species. EROD activity was increased more in rats, while phenacetin deethylase activity, PEROD, and nifedipine oxidase activity were increased by a greater amount and/or at lower doses in mice.

Literature studies of STP metabolism in rats (Zhang et al., 1990) and monkeys (Lin and Levy, 1983) were described in the NDA. Following administration of a single oral dose (200 mg/kg) to male S-D rats, a total of 15 metabolites (accounting for 44% of the administered dose collected over 48 hr) were identified in urine by GC/MS (Figure III.C.1), while only unchanged drug (accounting for a further 12.8% and 23.5% of the dose in two rats) was present in feces. Most of the metabolites resulted from one principal metabolic pathway: cytochrome P450-mediated cleavage of the methylenedioxy ring to yield catechol derivatives. A parallel metabolic pathway resulted in the formation of alcohols on the tertiary butyl group (structure V). Only two metabolites were not previously found in humans (Figure III.C.2, from Moreland et al., 1986): carboxylic acid derivatives (XIV and XVI) probably resulting from further oxidation of the corresponding primary alcohols.

Figure III.C.1 Urinary metabolites (II-XVI) of STP (I) found in rat urine

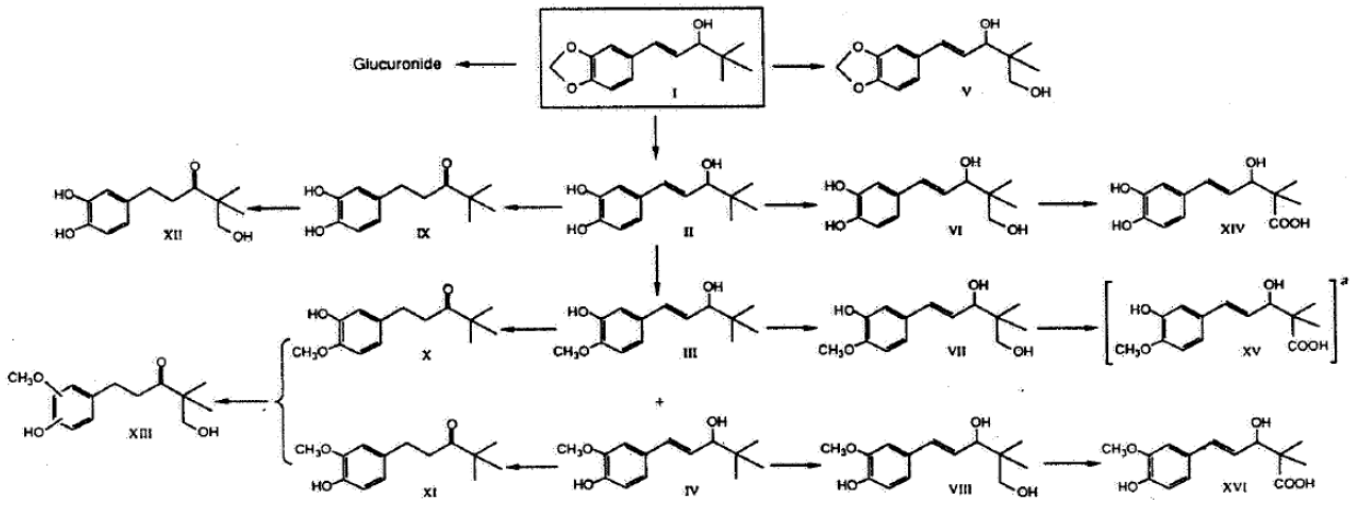
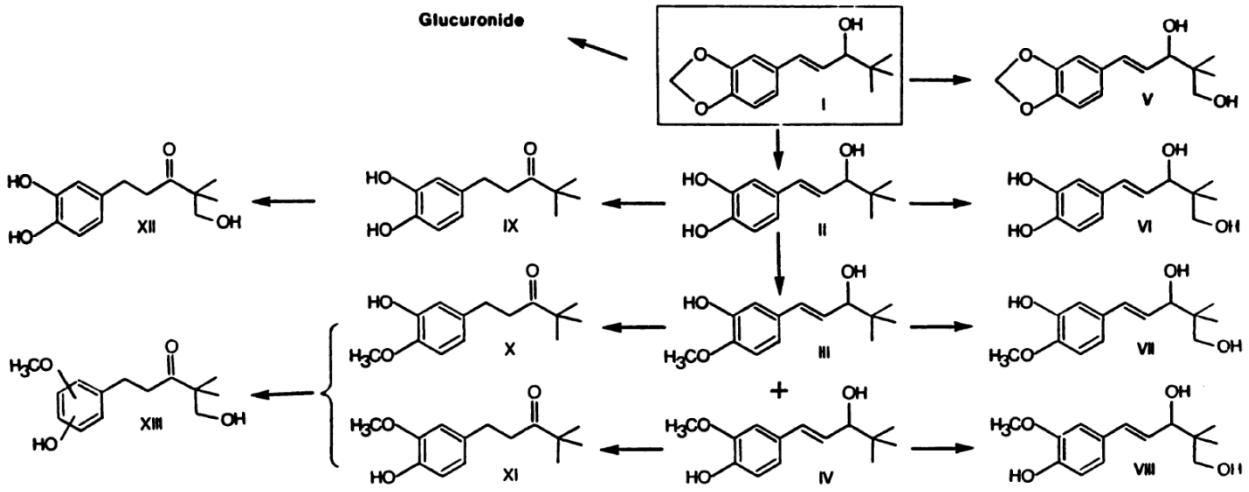
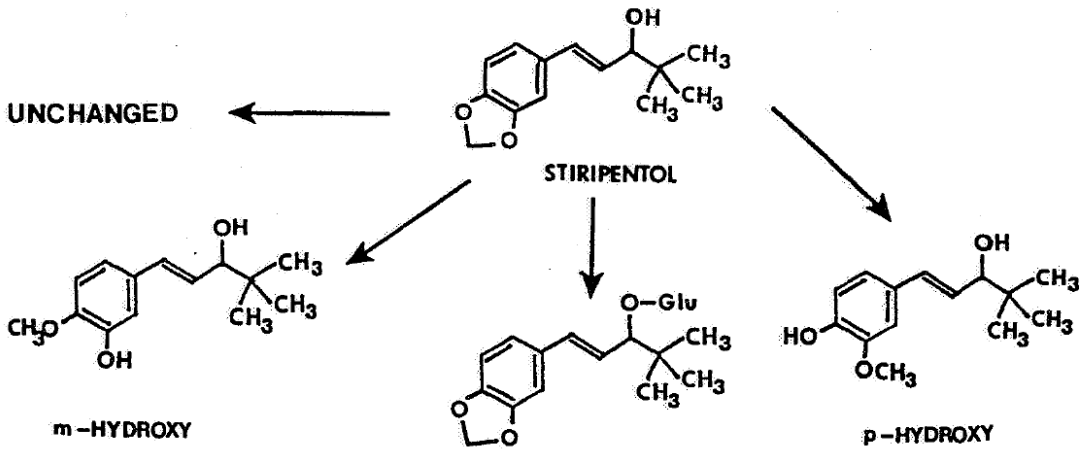


Figure III.C.2 Structures of stiripentol (I) and its metabolites (II-XIII) in human urine



Based on measurement of urinary metabolites in rhesus monkeys (not used in toxicity testing), STP was primarily metabolized via a glucuronidation pathway (30-40% of dose). Two metabolites resulting from the ring opening of the methylenedioxy moiety, p-hydroxy and m-hydroxy (Figure III.C.3), were also detected in monkey urine and had previously been identified in rats (Pieri et al., 1982). The p-hydroxy metabolite was shown to have some anticonvulsant activity in animal models, although it was much less potent than parent, while the m-hydroxy metabolite was inactive (Astoin et al., 1978). No information on plasma metabolites in animals or humans was provided.

Figure III.C.3. Ring opening metabolites of STP in rhesus monkey urine (from Lin and Levy, 1983)



STP is a racemate consisting of enantiomers determined to be (R)-(+) and (S)-(-). Chiral inversion of (R) to (S)-STP was found following oral administration to male S-D rats (Zang et al., 1994): after administration of (S)-STP, the plasma contained only that enantiomer (AUC S/R ratio = 47.7); when (R)-STP was given, both enantiomers were detected in plasma yielding an AUC S/R ratio = 0.86. This finding was explained by conversion of (R)-STP to its antipode. Also, when racemic drug was administered, (S)-STP was predominant in plasma with an AUC S/R ratio of 3.32. Brain distribution and CYP inhibition showed no differences between the enantiomers. In an earlier study (Shen et al, 1992), enantioselectivity was observed with respect to both the anticonvulsant activity and elimination kinetics of this compound. (R)-STP was 2.4 times more potent than its antipode against PTZ-induced clonic seizure (brain EC50 of 15.2 ug/g versus 36.1 ug/g). The (R)-enantiomer was eliminated more rapidly than the (S)-enantiomer, as reflected in a higher plasma clearance (1.64 L/h/kg versus 0.557 L/h/kg) and a shorter half-life (2.83 h versus 6.50 h).

D. Excretion

The only excretion study conducted by the sponsor examined the transfer of STP into milk in lactating goats. In that study, single dose or repeated doses (7 days) of STP (oral gavage dose of 200 mg/kg in 1% CMC) passed rapidly into the milk, and the M/P ratio was ~1.

Based on studies reported in the literature (Zhang et al., 1990 and Lin and Levy, 1983), the fraction of an STP dose appearing unchanged in urine was less than or equal to 1% in rat and monkey, consistent with elimination by metabolism. Following administration of a single oral dose (200 mg/kg) to male S-D rats, 44% of the administered dose was detected in urine, while 12.8-23.5% was present in feces.

IV. TOXICOLOGY

A. Subchronic toxicity

1. Stiripentol toxicity study for 13 weeks by oral administration (gavage) to mice (Biocodex Study Number: BC.231/GB, conducted [redacted] (b) (4), [redacted] (b) (4) report dated 5/17/91, GLP)

a. Methods

STP (lot 106) was administered to mice (Crl: CD-1 (ICR) BR; 10/sex/grp + 15/sex/grp TK) at oral (gavage) doses of 0 (vehicle: 0.5% carboxymethylcellulose), 60, or 800 mg/kg for 13 weeks. Clinical signs were recorded and mortality was checked twice a day. Body weight and food consumption were recorded once a week. Hematology (week 12), clinical chemistry evaluations (week 13), organ weights, and macroscopic and microscopic (full panel from C and HD and premature decedents) pathology examinations were performed (termination). During week 12, TK animals (3/sex/group/timepoint) were sampled before treatment and 1, 2, 6, and 24 hours after dosing. No dose justification was provided. This study was performed to determine doses for a mouse carcinogenicity study.

b. Results

i. Mortality and Clinical Observations

There were 1 LD and 6 HD deaths; however, all but 2 HD (1M, 1F) deaths, for which the cause could not be established, were determined to be due to gavage error. For the 2 HD deaths, a relationship to drug could not be ruled out.

ii. Body Weight

There were no effects on body weight gain, body weight, or food consumption.

iii. Clinical Pathology

There were no drug-related changes in hematology parameters. Clinical chemistry changes consisted of slightly (but SS) increased serum cholesterol in HD males.

iv. Necropsy

At necropsy, liver weights were increased (40-60%) in both sexes at the HD and accentuated lobular pattern of the liver was observed at the HD (3/10 males and 1/10 females).

Microscopically, an increase in the incidence and severity of hepatocyte hypertrophy (without hepatic cell degeneration or necrosis) was observed at the HD (9/10 males, 10/10 females).

v. Toxicokinetics

TK parameters were not calculated, but peak serum STP levels seen at 1 hr were 15.5 and 58.3 µg/mL in males and 13.6 and 63.2 ug/mL in females at the LD and HD, respectively.

c. Conclusions

Oral (gavage) administration of STP (0, 60, or 800 mg/kg/day) to CD-1 mice for 13 weeks resulted in possible drug-related deaths and adaptive liver changes (increases in cholesterol, liver weight, and hepatic cell hypertrophy) at the HD (Cmax ~ 60 ug/mL).

2. Subchronic toxicity study for 4 weeks by repeated oral administration in cynomolgus monkeys (Biocodex Study Number: BC.182/GB, conducted [redacted] (b) (4) report dated 11/29/89, GLP)

a. Methods

STP (lot 101) was administered to cynomolgus monkeys (2/sex/grp) at oral (gavage) doses of 0 (vehicle: 1% carboxymethylcellulose), 100, 300, or 900 mg/kg/day (5 mL/kg) for 4 weeks. Clinical signs and mortality were checked daily. Body weight and food consumption were recorded once a week. Other endpoints consisted of hematology, clinical chemistry, ECG, ophthalmologic examinations, organ weights, and macroscopic and microscopic pathology (full panel in all animals). Blood sampling for plasma drug level measurements was performed on days 1 and 28 predosing and 1.5, 2, 2.5, 3, and 24 hours after dosing. According to the study report, "dose levels were determined in agreement with the Sponsor, according to previous toxicology data," but it was not clear what data were being referred to since no other monkey studies besides the chronic toxicity study were submitted.

b. Results

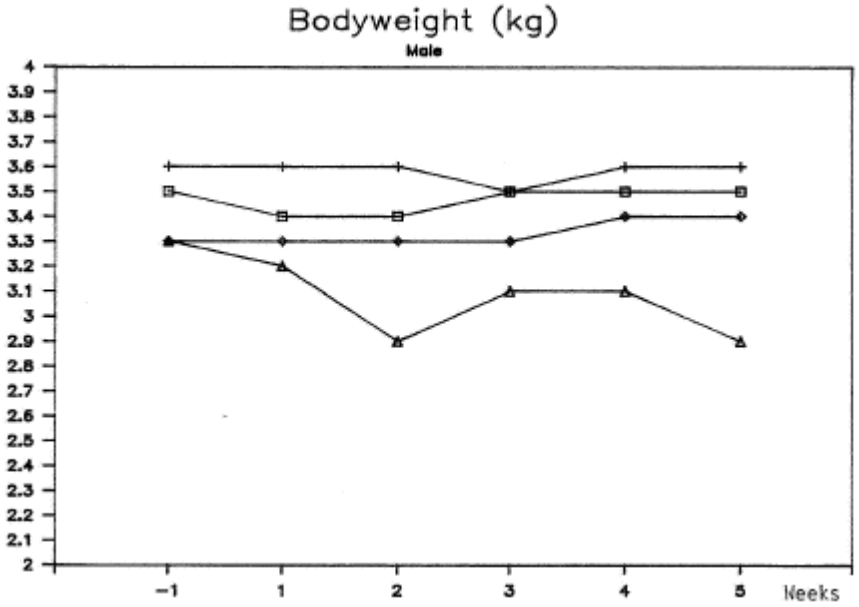
i. Mortality and Clinical Observations

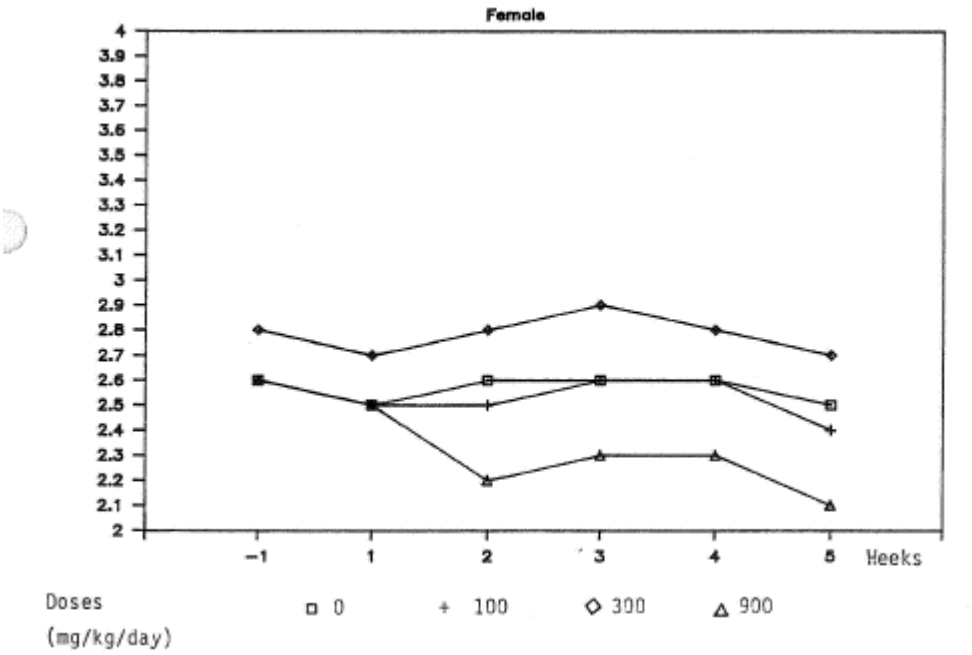
There was 1 HD female death. Marked hypotonia and sedation were noted in this animal prior to death, but the death was attributed to tubular nephrosis observed microscopically. Hypotonia (slight to marked) in the other HD female was the only clinical sign noted.

ii. Body Weight

Body weight gain was decreased in HD male and females (9 and 12% loss, respectively) during the first week of treatment but appeared to recover after that, although HD animals never attained their original weights (Figure IV.A.1.1).

Figure IV.B.1.1





iii. ECG

There were no ECG changes due to drug.

iv. Ophthalmology

There were no drug-related effects.

v. Clinical Pathology

There were no drug-related changes in hematology parameters. Clinical chemistry changes consisted of an increase (70% compared to predose) in blood urea in 1 HD male. This was thought to be related to the tubular nephrosis seen in this animal. No drug-related urinalysis changes were found.

vi. Necropsy

Increases (30-50%) in absolute and/or relative liver weights were found in HD males and females.

Focal tubular nephrosis (moderate to marked) was found in 1 HD male at terminal necropsy and the HD female that died on day 19 (Table IV.B.1.1).

Table IV.B.1.1

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------------------------------------------------------------------------
TEST ARTICLE   : STIRIPENTOL (D 306)                PATHOL. NO.: 04895 GER
TEST SYSTEM    : MONKEY, 4 weeks, Oral              DATE       : 26-MAY-89
SPONSOR        : BIOCODEX                           PDS PATHDATA SYSTEM TM
------------------------------------------------------------------------
NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
------------------------------------------------------------------------
                      DOSE GROUP:      T         A         B         C
                             SEX:    M   F     M   F     M   F     M   F
ORGAN/FINDING          NO.ANIMALS:   2   2     2   2     2   2     2   2
------------------------------------------------------------------------
KIDNEYS                 NO. EXAM.:   2   2     2   2     2   2     2   2
- MONO.CELL AGGREG.                  2   1     1   1     2   2     2   1
- MINERALISATION                                1                       
- EOSINOPHILIC CASTS                                                  1
- CICAT.TISSUE FORMAT.                              1                 
- FOC.TUBUL.NEPHROSIS                                                 1   1
- LEUCO.TUBULAR LUMEN.                                                1   1
- PERIGLOMERULAR FIBR.                             1                 
- TUBULAR DILATATION                                                      2
- TUBULAR CELL PIGMENT                                                    1
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vii. Toxicokinetics

After the first dose, Cmax was reached at 1.5 h when plasma levels were 5. 5, 10.1, and 20.5 ug/mL at the LD, MD, and HD, respectively. The Cmin and Cmax values after 28 days of dosing are shown in Table IV.B.1.2.

Table IV.B.1.2. Plasma STP levels in on Day 28 of 4-week monkey toxicity study

Plasma levels (ug/ml)	Doses (mg/kg/day) 100	300	900
C min (1)	1.9 ± 2.0	12.0 ± 7.8	36.7 ± 10.4 (2)
C at 1.5 h	11.5 ± 6.1	23.8 ± 3.9	78.0 ± 0.7 (2)

(1) calculated with the predose values (time = 0) and the values at time 24 h

(2) calculated excluding the value of animal D60608 which confirmed to be a low responder and animal D60618 which was dead

c. Conclusions

Oral (gavage) administration of STP (0, 100, 300, or 900 mg/kg/day) to cynomolgus monkeys for 4 weeks resulted in death (1 female), clinical signs (hypotonia, sedation), bodyweight loss, increased blood urea level, increased liver weight, and renal tubular nephrosis at the HD.

B. Chronic toxicity

1. 26-week toxicity study by oral route (gavage) in rats (Biocodex Study Number: BC.232/GB, [redacted] (b) (4) report dated 12/23/94, GLP)

a. Methods

Sprague-Dawley (Crl: CD(SD) BR) rats (20/sex/group main, 10/sex/grp TK) were administered STP (lot 106) at doses of 0 (vehicle: 0.5% aqueous solution of carboxymethylcellulose), 80, 220, or 800 mg/kg by oral gavage (5 mL/kg) once daily for 26 weeks. Observations included clinical signs, body weight, ophthalmology, clinical pathology (weeks 13 and 26), and gross and microscopic (full panel of tissues from C & HD and premature decedents; lung, liver, and kidney from LD & MD) pathology evaluations. Blood was collected for plasma drug level measurements 1, 2, 4, 6, and 24 hr after dosing at 4 and 26 weeks in LD and HD groups only. According to the study report, "dose levels were determined in agreement with the Sponsor, according to previous toxicology data," but it was not clear what data were being referred to. The only other repeated-dose rat studies submitted were two non-GLP studies: one, a 6-month study in Wistar rats with oral (gavage) doses of 0 (1% CMC), 30, 60, or 300 mg/kg, which was only briefly described (data not included in report), showed decreased BWs in MD and HD females and increased liver weights at the HD in both sexes but reportedly did not indicate any clinical pathology or histopathological effects; the other, a 21-day study in S-D rats with oral (gavage) doses of 0 (0.5% CMC), 80, 220, or 800 mg/kg (BC.266/EN) only examined liver weights and found that they were dose-dependently increased by STP (33 and 47% at HD).

b. Results

i. Mortality and Clinical Observations

There were 3 deaths considered possibly drug-related: 1 MD male (No. J23252) found dead on day 188 (week 27) after clinical signs of dyspnea from week 26, loud breathing from week 27, and regurgitation on weeks 26 and 27; 1 HD female (123411) found dead on day 102 (week 15), just after dosing, without presenting clinical signs other than excessive salivation (ptyalism); and 1 HD female (J23426) killed prematurely on day 51 (week 8) due to poor clinical condition (emaciation, piloerection, round back, sedation, dyspnea, and half-closed eyes). There were no macroscopic or microscopic findings in these animals that were thought to have contributed to death, but a relationship to treatment could not be ruled out.

Signs of poor clinical condition consisting of dyspnea loud breathing, piloerection, round back, emaciation, soiled urogenital area and/or sedation was noted in 1/20 MD males and 2/30 males and 5/30 females at the HD. In the MD male and 1 HD female, these findings preceded the death or the sacrifice of the animal. For the other animals, the duration of the clinical signs was variable (between a few days and several weeks); in some cases they disappeared in the course of the treatment period. Ptyalism was also observed with a dose-related incidence and severity in all treatment groups (all males and females affected at HD). This clinical sign lasted from a few days (LD) to the entire treatment period (most HD females).

ii. Body Weight

Over the treatment period, body weight (BW) gain was decreased (8, 21, and 25%) in MD males and HD males and females, respectively, resulting in BW decreases of 6, 15, and 14%, respectively, at week 26 (Figure IV.B.1.1-2).

Figure IV.B.1.1

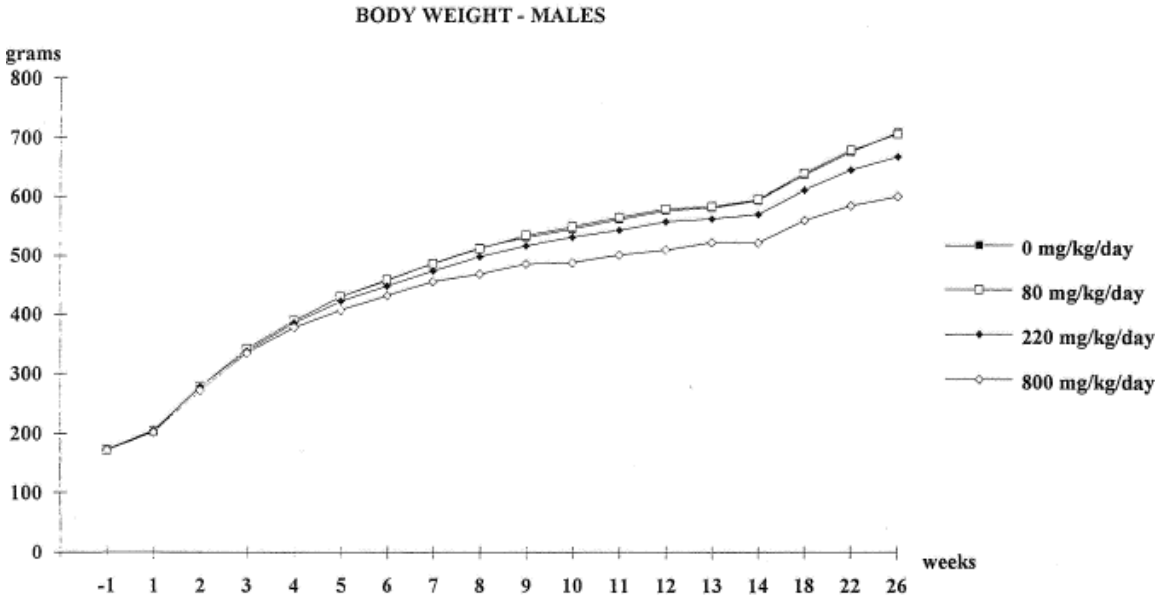
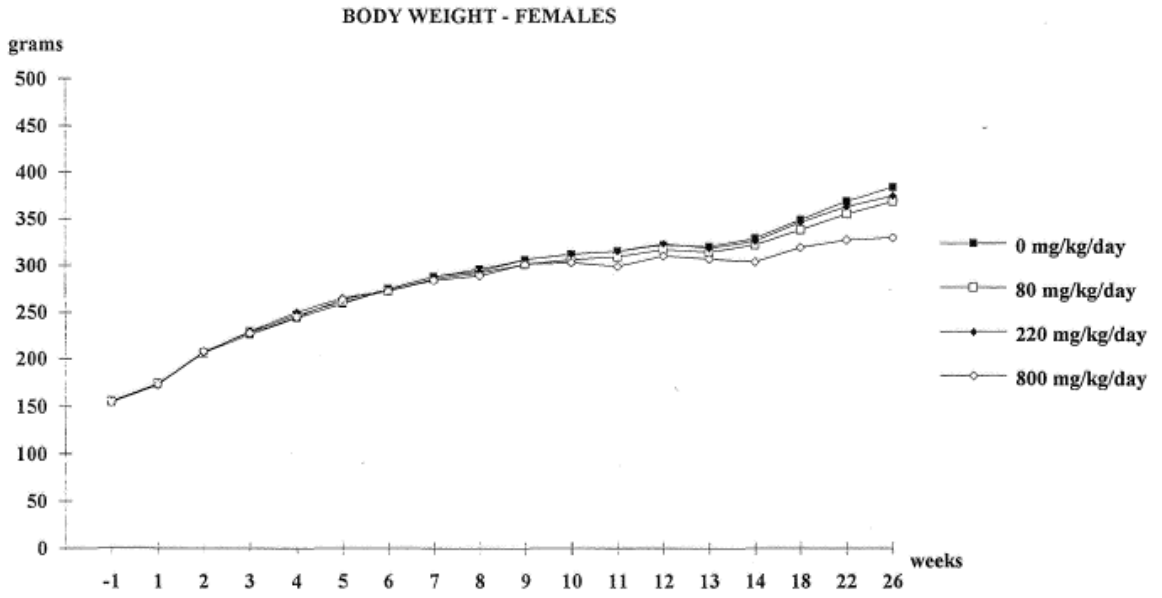


Figure IV.B.1.2



iii. Ophthalmoscopy

There were no drug-related effects.

iv. Clinical Pathology

There were no clearly drug-related changes in hematological values.

Dose-related (D-R) decreases in glucose (up to 32%) and increases in urea (54%), bilirubin (2X), total protein (17%), and cholesterol (102%) values were seen in both sexes at 13 and 26 weeks (Tables IV.B.1.1-2). Changes in electrolytes (↓Na+, Cl-; ↑K+, iPhos, Ca++) were also seen in treatment groups (SS at HD) but were not always strictly D-R. Liver enzymes were decreased in treated groups. The effect on urea (electrolytes not mentioned in report) was thought to correlate with histopathological changes noted in the kidneys (tubular nephrosis) and the changes in glucose and cholesterol with hepatic cell hypertrophy observed among these animals.

Table IV.B.1.1 Clinical chemistry (males)

Sex: Male
Time: Week 26

Dose (mg/kg/d)		0	80	220	800
Na+ mmol/l	M (1)	145.3	144.0 **	144.0 **	144.2 *
	SD	1.13	0.69	0.86	0.72
	n	10	10	10	10
K+ mmol/l	M (1)	3.53	3.57	3.87 *	3.82
	SD	0.368	0.192	0.275	0.338
	n	10	10	10	10
Cl- mmol/l	M (1)	103.6	103.2	102.9	102.1 *
	SD	1.41	1.27	1.18	1.25
	n	10	10	10	10
Ca++ mmol/l	M (1)	2.69	2.63	2.69	2.73
	SD	0.061	0.060	0.067	0.054
	n	10	10	10	10
I.PHOS mmol/l	M (1)	1.95	1.74 **	1.96	2.20 **
	SD	0.171	0.145	0.123	0.131
	n	10	10	10	10
GLUC mmol/l	M (1)	8.55	7.92	7.84	5.83 **
	SD	1.100	0.642	0.934	0.614
	n	10	10	10	10
UREA mmol/l	M (1)	4.5	4.7	5.4 *	6.4 **
	SD	0.47	0.43	0.76	0.90
	n	10	10	10	10
CREAT µmol/l	M (1)	47	46	44	47
	SD	4.6	4.8	6.1	6.0
	n	10	10	10	10

PROT	M (1)	74	76	77	80 **
g/l	SD	2.3	3.8	3.3	4.5
	n	10	10	10	10
ALB	M	32	32	32	33
g/l	SD	0.6	0.7	1.3	2.0
	n (B)	10	10	10	10
A/G	M (1)	0.76	0.74	0.72	0.71 *
1	SD	0.048	0.044	0.038	0.037
	n	10	10	10	10
TOT.BIL.	M (1)	1	1	1	2
µmol/l	SD	0.5	0.3	0.5	0.5
	n	10	10	10	10
CHOL	M (1)	1.6	1.8	2.0 *	2.3 **
mmol/l	SD	0.40	0.34	0.26	0.40
	n	10	10	10	10
TRIG	M (1)	1.19	1.28	0.94	1.03
mmol/l	SD	0.416	0.400	0.456	0.496
	n	10	10	10	10
ALP	M (1)	124	119	110	87 **
IU/l	SD	28.9	20.2	14.6	18.8
	n	10	10	10	10
ASAT	M (3)	71	50 **	75	60
IU/l	SD	13.7	7.6	26.2	15.1
	n (B)	10	10	10	10
ALAT	M (1)	34	18 *	29	23
IU/l	SD	11.4	5.7	17.4	10.1
	n	10	10	10	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests

(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

Table IV.B.1.2 Clinical chemistry (females)

Sex: Female
Time: Week 26

Dose (mg/kg/d)		0	80	220	800
Na+	M (1)	143.9	144.1	143.5	143.2
mmol/l	SD	1.67	1.57	1.47	1.27
	n	10	10	10	10
K+	M (1)	3.38	2.95	3.35	3.47
mmol/l	SD	0.461	0.388	0.473	0.491
	n	10	10	10	10
Cl-	M (1)	104.2	102.5	102.9	101.0 *
mmol/l	SD	2.49	3.41	2.59	1.50
	n	10	10	10	10
Ca++	M (1)	2.76	2.77	2.76	2.89 **
mmol/l	SD	0.076	0.105	0.115	0.075
	n	10	10	10	10
I.PHOS	M (1)	1.32	1.49	1.53	1.70 **
mmol/l	SD	0.183	0.185	0.324	0.142
	n	10	10	10	10
GLUC	M (1)	7.76	7.87	7.26	6.49 *
mmol/l	SD	0.545	1.362	1.131	1.130
	n	10	10	10	10
UREA	M (1)	4.6	5.9 *	5.5	6.2 **
mmol/l	SD	0.56	0.91	1.04	1.55
	n	10	10	10	10
CREAT	M (3)	49	58	51	50
µmol/l	SD	2.9	9.7	4.3	12.0
	n (B)	10	10	10	10

PROT g/l	M (1)	81	84	86	91 **
	SD	4.0	5.7	4.5	4.6
	n	10	10	10	10
ALB g/l	M (1)	38	40	40	39
	SD	2.9	3.1	3.1	2.5
	n	10	10	10	10
A/G 1	M (1)	0.90	0.89	0.89	0.77 **
	SD	0.080	0.085	0.080	0.047
	n	10	10	10	10
TOT.BIL. µmol/l	M (1)	1	2	2	2 *
	SD	0.5	0.6	0.7	0.6
	n	10	10	10	10
CHOL mmol/l	M (1)	2.7	3.2	3.4	5.4 **
	SD	0.63	1.23	1.11	1.08
	n	10	10	10	10
TRIG mmol/l	M (3)	0.93	0.67	0.92	0.75
	SD	0.341	0.351	0.883	0.321
	n (B)	10	10	10	10
ALP IU/l	M (1)	52	62	54	46
	SD	19.2	22.0	19.7	15.3
	n	10	10	10	10
ASAT IU/l	M (3)	118	65	62	46 **
	SD	60.3	25.8	19.3	11.5
	n (B)	10	10	10	10
ALAT IU/l	M (3)	52	21	20 *	16 **
	SD	31.0	9.8	10.3	7.8
	n (B)	10	10	10	10

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests
(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smimov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

Urinalysis found an increase in the incidence and/or severity of urinary protein in LD and MD females and in HD males and females, which was also consistent with the histopathological changes in the kidneys (Table IV.B.1.3).

Table IV.B.1.3 Urinary protein in chronic rat toxicity study

Doses (mg/kg/day)	0	80	220	800
Males				
grade 0	3/10	2/10	2/10	3/10
grade 1	5/10	6/10	4/10	3/10
grade 2	1/10	1/10	2/10	3/10
grade 3	1/10	0/10	2/10	1/10
grade 4	0/10	1/10	0/10	0/10
Females				
grade 0	7/10	1/10	2/10	1/10
grade 1	1/10	2/10	3/10	2/10
grade 2	1/10	2/10	0/10	0/10
grade 3	0/10	1/10	1/10	3/10
grade 4	1/10	4/10	4/10	4/10

Grade 0: negative Grade 1: 0.3 g/l Grade 2: 1 g/l
Grade 3: 3 g/l Grade 4: ≥ 20 g/l

v. Necropsy

Dose-related (and SS) increases in liver and kidney weights (absolute and/or relative) were seen in both sexes at the MD and HD.

Macroscopic –

Liver enlargement was noted in 4/20 males and 5/20 females at the HD.

Microscopic (no signed and dated pathology report) –

Moderate to marked centrilobular hepatocyte hypertrophy was seen in males and females at the MD and HD (Table IV.B.1.4). There was no evidence of degenerative/necrotic changes, however.

Table IV.B.1.4

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
IN MG/KG/DAY: 1=0; 2=80; 3=220; 4=800

DOSE GROUP:	1		2		3		4	
SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING NO.ANIMALS:	20	20	20	20	20	20	20	20
LIVER NO. EXAM.:	20	20	20	20	20	20	20	20
- CENTRIL.H.C.HYPERTR.					18	20	16	20
- INTRACYT.CONCEN.INC.							1	
- PERICHOLANGITIS	1	2		1	1			1
- EXTRAMEDUL.HAEMATOP.	5	5	1		2		1	1
- MONO.CELL AGGREGAT.	18	17	16	18	12	14	16	12
- MICROGRANULOMA	2	2	3	5	2	5		2
- MULTIF.COAG.H.C.NEC.	2	1						
- FOC.COAG.H.CEL.NECR.	1							
- AREA OF H.C.NEC.FIB.			1					
- PELIOSIS.	2							
- MACROPH.LAD.YEL.PIG.		1						
- TENSION LIPIDOSIS.	4	3	1	2	1	7	1	7
- ALTERED CEL.FOC.,AC.						1		
- ARTERITIS.					1			
- INTERLOB.FIBROPLASIA						1		
- PERIVASCULITIS.	1			1				
- STEATOSIS.	1	5	1	2		2	1	1
- CHRONIC CAPS.INFLAM.				1				

In the kidneys (Table IV.B.1.5), tubular nephrosis (graded slight to moderate) was increased at the MD (6/20 males and 8/20 females) and HD (4/20 males and 8/20 females). This was associated with increase incidences of tubular basophilia in these groups (12 males and 9 females at MD; 15 males and 12 females at HD vs 8 males and 3 females in C group). In addition, increased incidences and severity of acidophilic globule accumulation in the cortical tubular epithelium were observed in treated males (11 MD and 12 HD vs 5 C).

Table IV.B.1.5

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
IN MG/KG/DAY: 1=0; 2=80; 3=220; 4=800

	DOSE GROUP:	1		2		3		4	
	SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO.ANIMALS:	20	20	20	20	20	20	20	20
KIDNEYS	NO. EXAM.:	20	20	20	20	20	20	20	20
- DILATED PELVIS		1						2	
- MINERALISATION.			2						
- INTERS.MONO.CEL.AGGR		6	7	11	5	5	9	9	2
- TUBULAR BASOPHILIA		8	3	7	4	12	9	15	12
- TUBULAR DILATATION		2	3		1			1	2
- TUBULAR NEPHROSIS.						6	8	4	8
- ACI.GLOB.COR.TUB.EP.		5	1	5		11	1	12	4
- GLOMERULONEPHRITIS.		1			1		1		1
- TUBULAR LIPOFUSCIN.		1							
- CHR.TUB.INTER.NEPHR.					1				
- ACIDOPHILIC CASTS.		1	3	2	4	2	9	2	9
- RENAL CYST.			2						1
- PERIGLOMERULAR FIBR.		2						3	
- PERITUBULAR FIBROSIS		2	1			3	1	5	2
- CONTRACTED GLOM.TUF.			1			1			4
- LEUCOHISTIOCYT.PYEL.		1			1				
- HYPERPL.,PELV.EPITH.		2						1	
- FOC.NECR.,PELV.EPIT.		2							
- GRAVEL, RENAL PELVIS								1	

vi. Toxicokinetics

The sponsor did not calculate TK parameters but provided the following figures showing plasma levels of STP as a function of time at the LD and HD. Peak plasma STP levels (seen at 6 hr) at week 26 were approximately 50 ug/mL in both sexes at the HD. However, at the no-effect (LD) dose, peak plasma levels (at 2 hr) were approximately 4 and 11 ug/ml in males and females, respectively.

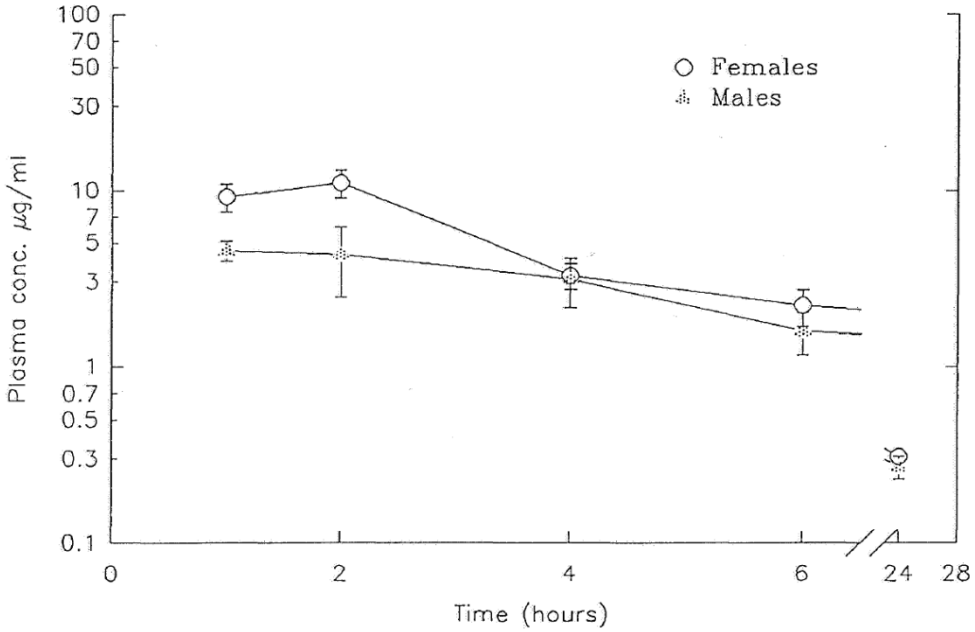


Figure IV.B.1.3. Plasma levels of STP in LD group at 26 weeks

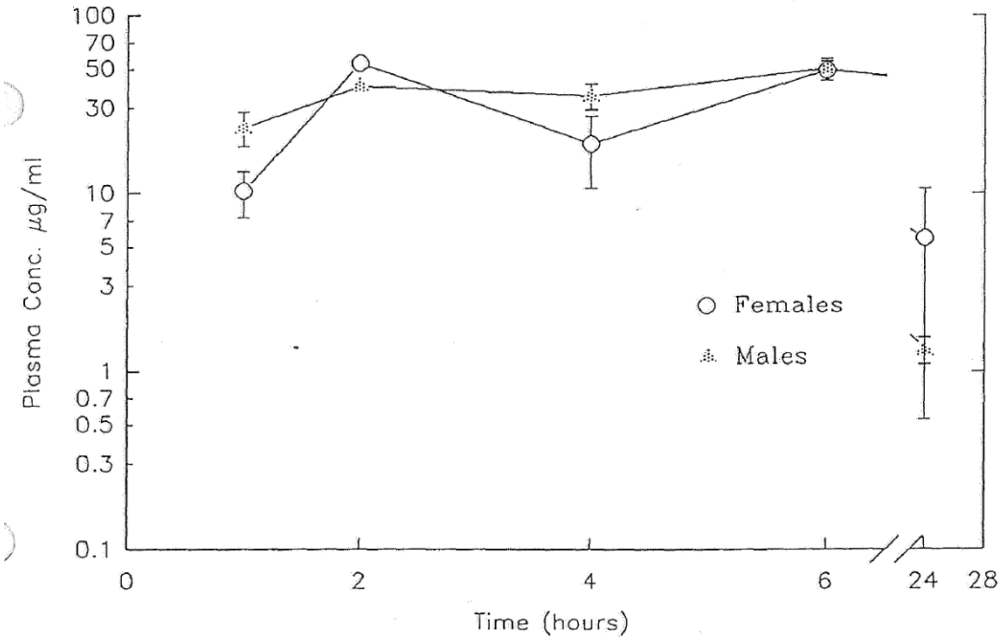


Figure IV.B.1.4. Plasma levels of STP in HD group at 26 weeks

c. Conclusions

Oral (gavage) administration of STP (80, 220, and 800 mg/kg/day) to S-D rats for 26 weeks resulted in deaths, clinical signs, decreased BW, clinical chemistry, urinalysis, and macroscopic and microscopic evidence of adaptive liver changes (hepatocellular hypertrophy) and kidney toxicity (tubular nephrosis) at the MD and HD.

2. Stiripentol (D 306): toxicity study for 26 weeks by repeated oral administration in cynomolgus monkeys followed by a 4-week recovery period (Biocodex Study Number: BC.183-1/GB, [redacted] (b) (4) report dated 3/21/91, GLP)

a. Methods

STP (lot 101) was administered to cynomolgus monkeys (4/sex/grp main study, 2/sex/grp recovery) at oral (gavage) doses of 0 (vehicle: 1% carboxymethylcellulose), 100, 250, or 600 mg/kg/day (5 mL/kg) for 26 weeks. Clinical signs and mortality were checked daily. Body weight and food consumption were recorded once a week. Other endpoints consisted of hematology, clinical chemistry, ECG, ophthalmologic examinations, organ weights, and macroscopic and microscopic pathology (full panel in all animals). Blood samples for TK were taken at week 9, before and 1, 2, 4, 6, and 24 hours after dosing. Doses were based on the results of the 4-week toxicity study in cynomolgus monkeys (above).

b. Results

i. Mortality and Clinical Observations

There were no drug-related deaths or clinical signs. Deaths of 1 C male and 1 LD female on days 49 and 13 were attributed to dosing accidents.

ii. Body Weight

Body weight (BW) gain and BW appeared to be decreased in HD animals, but SS was not reached (Figure IV.B.2.1).

iii. Ophthalmoscopy (predose and weeks 13 and 26)

There were no drug-related changes in ophthalmic findings.

iv. ECG (predose and weeks 13 and 26)

There were no drug-related effects on the EEG.

v. Clinical Pathology (predose and weeks 4, 12, and 26)

Dose-related decreases in RBC parameters were observed in treated groups of both sexes at each interval (SS seen at all doses at some intervals; Table IV.B.2.1). No evidence of reversibility was found after the recovery period. WBCs were also dose-dependently decreased but the differences were not SS.

There were no clear drug-related effects on clinical chemistry or urinalysis values. The increased urea seen in the 4-week monkey study at a higher dose and in the 26-week rat study was not consistently seen in this study, although blood urea was somewhat elevated in HD females at 12 and 26 weeks. In addition, ALP activity tended to be decreased in treated groups, particularly in HD males at 12 and 26 weeks.

Table IV.B.2.1.

Study : 4946 TCP
Test substance : STIRIPENTOL (D 306)
Sex : Male
Time : Week 12

Dose (mg/kg/d)		0	100	250	600
WBC	M (1)	14.0	13.9	11.5	9.9
G/l	SD	2.94	3.05	1.61	2.93
	n	5	6	6	6
RBC	M (1)	6.80	6.79	6.37	5.92 *
T/l	SD	0.358	0.355	0.474	0.576
	n	5	6	6	6
HB	M (1)	13.5	12.2 *	11.5 **	11.2 **
g/dl	SD	0.29	1.02	0.50	0.71
	n	5	6	6	6
PCV	M (1)	0.46	0.42 *	0.40 **	0.39 **
l	SD	0.008	0.031	0.023	0.024
	n	5	6	6	6
MCV	M (1)	67	62	63	66
fl	SD	2.2	3.6	4.6	3.4
	n	5	6	6	6
MCH	M (1)	19.9	18.0 *	18.1 *	19.1
pg	SD	0.86	1.15	1.12	1.40
	n	5	6	6	6
MCHC	M (1)	29.6	29.1	28.8	28.9
g/dl	SD	0.41	0.52	0.78	0.84
	n	5	6	6	6
PLAT	M (1)	418	502	443	448
G/l	SD	112.2	62.9	105.3	95.8
	n	5	6	6	6

SIGNIFICANCE OF THE DIFFERENCE BETWEEN TREATED AND CONTROL GROUPS

* P<0.05
** P<0.01
(1) : DUNNETT TEST
(2) : MANN-WHITNEY TEST

SAMPLE DISTRIBUTION-RELATIVE TESTS

(B) BARTLETT TEST P<0.01
(F) FISHER TEST P<0.05
(K) KOLMOGOROV-SMIRNOV TEST P<0.01
(L) LOGARITHMIC TRANSFORMATION

Study : 4946 TCP
Test substance : STIRIPENTOL (D 306)
Sex : Female
Time : Week 12

Dose (mg/kg/d)			0	100	250	600
WBC	M	(1)	13.1	10.9	10.8	10.7
G/l	SD		2.92	3.82	2.22	3.36
	n		6	5	6	6
RBC	M	(1)	6.22	6.23	5.92	5.64
T/l	SD		0.492	0.669	0.342	0.639
	n		6	5	6	6
HB	M	(1)	12.0	11.3	10.7	10.3 *
g/dl	SD		0.98	1.18	0.73	1.34
	n		6	5	6	6
PCV	M	(1)	0.42	0.40	0.38	0.36 **
l	SD		0.027	0.028	0.021	0.040
	n		6	5	6	6
MCV	M	(1)	68	65	64	64
fl	SD		3.6	5.3	4.8	4.4
	n		6	5	6	6
MCH	M	(1)	19.4	18.2	18.2	18.2
pg	SD		1.30	1.51	1.51	0.92
	n		6	5	6	6
MCHC	M	(1)	28.7	28.2	28.3	28.5
g/dl	SD		0.48	1.00	0.54	1.13
	n		6	5	6	6
PLAT	M	(1)	467	431	508	418
G/l	SD		114.7	134.2	162.8	84.0
	n		6	5	6	6

SIGNIFICANCE OF THE DIFFERENCE BETWEEN TREATED AND CONTROL GROUPS

* P<0.05
** P<0.01
(1) : DUNNETT TEST
(2) : MANN-WHITNEY TEST

SAMPLE DISTRIBUTION-RELATIVE TESTS

(B) BARTLETT TEST P<0.01
(F) FISHER TEST P<0.05
(K) KOLMOGOROV-SMIRNOV TEST P<0.01
(L) LOGARITHMIC TRANSFORMATION

vi. Necropsy

Macroscopic -

Increases in absolute and relative liver weights were found in MD and HD males at the end of the treatment but not after the 4-weeks recovery period. There were no macroscopic changes reported.

Microscopic (full panel of tissues examined; signed and dated pathology report not included) -

On microscopic examination, no changes considered drug-related were reported. Incidences of tubular dilatation sometimes accompanied by calcification and eosinophilic casts in the tubular lumen were found in comparable incidence and severity in the majority of both control and treated animals (Table IV.B.2.2). However, chronic tubulo-interstitial nephropathy was found in 1 male (1017) and 1 female (1066) from the MD group. These animals had peak levels somewhat higher than the group means (male 18.1 ug/mL, female 12.1 ug/mL) but not the highest levels in the group and not comparable to HD group. There were no liver findings, such as hepatocellular hypertrophy, to correlate with the increased liver weights.

Table IV.B.2.2. Microscopic kidney findings in 26-week monkey toxicity study (main study)

PATHOLOGY REPORT — PAGE : 12
SUMMARY TABLES — (b)(4) PROJECT: 4946 TCF

TEST ARTICLE : STIRIPENTOL (D 306) — PATHOL. NO.: 04946 GER
TEST SYSTEM : MONKEY, 26 WEEKS + 4 WEEKS, ORAL — DATE : 11-JUN-90
SPONSOR : BIOCODEX — PDS PATHDATA SYSTEM TM

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
FINAL SACRIF. IN MG/KG/D: T=0 A=100 B=250 C=600

	DOSE GROUP:	T		A		B		C	
	SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO.ANIMALS:	4	4	4	4	4	4	4	4
KIDNEYS	NO. EXAM.:	4	4	4	4	4	4	4	4
- INTERS.MONO.CELL AG.		2	1	2	2		1	1	1
- TUBULAR DILATATION.		4	3	4	2	2	3	3	4
- EOSINOPHILIC CASTS									1
- CALCIFICATION				1					1
- CHR.TUB-INT.NEPHROP						1	1		

vii. Toxicokinetics

Cmin and Cmax values at 9 weeks are shown in Table IV.B.2.2. AUCs were not calculated.

Table IV.B.2.2 Plasma levels of STP in 6-month monkey toxicity study (ug/mL)

Doses mg/kg/day	Sexes	Cmin (1)	C 1 h
100	male	0.9 ± 0.7	11.0 ± 4.0
	female	0.8 ± 0.8	11.2 ± 5.9
250	male	2.4 ± 1.4	16.5 ± 3.5
	female	1.3 ± 0.6	11.4 ± 2.9
600	male	8.4 ± 8.0	18.9 ± 3.4
	female	5.5 ± 4.3	20.4 ± 7.0

(1) calculated with the predose values (time=0) and the values at time 24 h.

c. Conclusions

Oral (gavage) administration of STP (0, 100, 250, or 600 mg/kg/day) to cynomolgus monkeys for 26 weeks resulted in dose-related decreases in RBC parameters at all doses and increased liver weights and possible renal toxicity (tubulo-interstitial nephropathy) at the MD and HD.

C. Genotoxicity

1. Mutagenicity evaluation Ames salmonella/microsome plate test (Biocodex Study No. BC.123-1 [redacted] (b) (4) report dated 1/15/81, QA but non-GLP)

The results of what appeared to be an adequate Ames test (tester strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100) conducted with STP (0.5 to 1000 ug/plate; no batch or lot number given) in the absence and presence of a metabolic activation system (rat S9) were negative.

2. Chromosomal analysis in vitro in CHO chinese hamster cells (Study No. BC.185/GB, [redacted] (b) (4) report dated 2/08/90, GLP)

After a preliminary cytotoxicity test, cells (2 cultures/concentration) were exposed to STP (0, 0 (DMSO), 10, 30, and 60 ug/mL without rat S9 and 0, 0 (DMSO) 30, 100, and 150 ug/mL with S9; Batch no. 101) for 4 hours. The mitotic index of the treated cells was equivalent to that of the controls. However, at the highest concentrations, both without and with S9, according to the report "many cells were round and refrigent," which indicated cytotoxicity. At the lower concentrations, no morphological alterations were observed. At the highest concentrations (60 and 150 ug/mL, without and with S9), the incidence of aberrant cells was significantly increased compared to the negative control (Tables IV.C.2.1-2). The positive controls performed as expected. The conclusion was that STP induced chromosomal aberrations (chromatid-type and chromosomal-type) at cytotoxic concentrations.

Table IV.C.2.1 Test without metabolic activation

Number of cells with aberrations per 200 analysed metaphases

Substance	Dose (mcg/ml)	Gap	Chromatid		Chromosome		MA	PU	Number of cells with aberrations		Percentage of cells with aberrations	
			Break	Exchange	Break	Exchange			with Gap	without Gap	with Gap	without Gap
-	-	6	1	0	0	0	2	0	9	3	4.5	1.5
DMSO	-	3	5	3	1	0	0	0	12	9	6.0	4.5
STIRI-PENTOL (D 306)	10	7	1	1	0	3	1	1	12	6	6.0	3.0
	30	1	2	1	0	2	0	0	6	5	3.0	2.5
	60	6	10	6	4	2	2	0	22	19	11.0	9.5*
MMS	52	15	28	22	2	3	2	1	56	49	28.0	24.5***

MA: Multiple aberrations
PU: pulverisation
Number of aberrations per 200 analysed metaphases: MA and PU are not included in the total
Number of cells with aberrations: MA and PU are included in the total
Statistical test used: X_2
*** : $p < 0.001$
* : $p < 0.05$

Table IV.C.2.2 Test with metabolic activation

Number of cells with aberrations per 200 analysed metaphases

Substance	Dose (mcg/ml)	Gap	Chromatid Break	Chromatid Exchange	Chromosome Break	Chromosome Exchange	MA	PU	Number of cells with aberrations with Gap	Number of cells with aberrations without Gap	Percentage of cells with aberrations with Gap	Percentage of cells with aberrations without Gap
-	-	5	2	3	0	2	1	0	12	8	6.0	4.0
DMSO	-	1	0	1	0	0	0	0	2	1	1.0	0.5
STIRI-PENTOL (D 306)	30	3	4	3	1	2	0	0	13	10	6.5	5.0
	100	7	4	1	0	0	0	0	12	5	6.0	2.5
	150	9	20	24	5	4	0	0	51	44	25.5	22.0***
CPA	30	17	34	23	4	5	2	0	66	58	33.0	29.0***

MA: Multiple aberrations
PU: pulverisation
Number of aberrations per 200 analysed metaphases: MA and PU are not included in the total
Number of cells with aberrations: MA and PU are included in the total
Statistical test used: X_2
*** : $p < 0.001$

3. HPRT gene mutation assay in V79 chinese hamster cells (Study No. BC.1861GB, (b) (4) (b) (4), report dated 2/5/90, GLP)

STP (batch # 101) was tested at concentrations of 0, 0(DMSO), 1, 3, 10, 30, and 100 ug/mL without rat S9 and 0, 0(DMSO), 3, 10, 30, 100, and 300 ug/mL with S9. No mutagenic effect was observed either without or with S9 at the concentrations used. The positive controls produced the expected increases in mutation frequencies.

4. Study of the genotoxic activity of the product stiripentol by chromosome aberration testing by metaphase analysis on cultured human lymphocytes cells (Study No. BC.187/EN, (b) (4) (b) (4), report dated 2/27/90, GLP)

When the potential clastogenic activity of STP (batch # 101) was evaluated in vitro in human lymphocytes in culture at up to cytotoxic concentrations (100 µg/mL in the presence of metabolic activation, 30 µg/mL in its absence) there was no increase in chromosomal aberrations. The positive and negative controls performed as expected.

5. Mutagenesis study using the micronucleus technique in the mouse (Study No. BC.137/GB, (b) (4), report dated 10/11/84, GLP)

When the potential mutagenic action of STP (batch # 84) was examined in the mouse micronucleus test after 2 daily oral (gavage) doses of 0 (1% gum tragacanth vehicle), 1250, or 2500 mg/kg, no increase in the number of micronuclei was seen. In the preliminary toxicity study, a dose of 5 g/kg caused mortality in 1/6 animals. Results obtained in the negative and positive (cyclophosphamide) control animals indicated a valid study.

D. Carcinogenicity

1. Potential tumorigenic effects in long-term administration (gavage) to mice for 78 weeks (Study no.: BC.234/GB [redacted] (b) (4) report dated 4/15/94, GLP)

a. Methods

STP (0 [0.5% CMC vehicle; T1], 0 [0.5% CMC vehicle; T2], 60, 200, or 600 mg/kg; lot # 106) was administered daily by oral gavage (10 mL/kg) for 78 weeks to CD-1 (Crl:CD-1(ICR)BR) mice (50/sex/grp + 5/sex/grp TK). Observations included clinical signs, mortality, palpation for masses, food consumption and body weight, hematology (weeks 52 and 78), and macroscopic and microscopic pathology examinations (full panel of tissues in C (T1) and HD and animals that died or were sacrificed prematurely; masses, lesions, and liver in all groups, including T2). Blood samples were taken 1 hour after dosing at 2 and 78 weeks for plasma drug level measurements.

Dose selection was based on the results of the 13-week oral (gavage) toxicity study in CD-1 mice (BC.231/GB, described above) in which possibly drug-related deaths (1M, 1F), increased liver weights, and hepatocellular hypertrophy were seen at the HD (800 mg/kg/day) in both sexes. (Carcinogenicity study protocol was not reviewed by the Exec-CAC.)

b. Results

i. Mortality and Clinical Observations

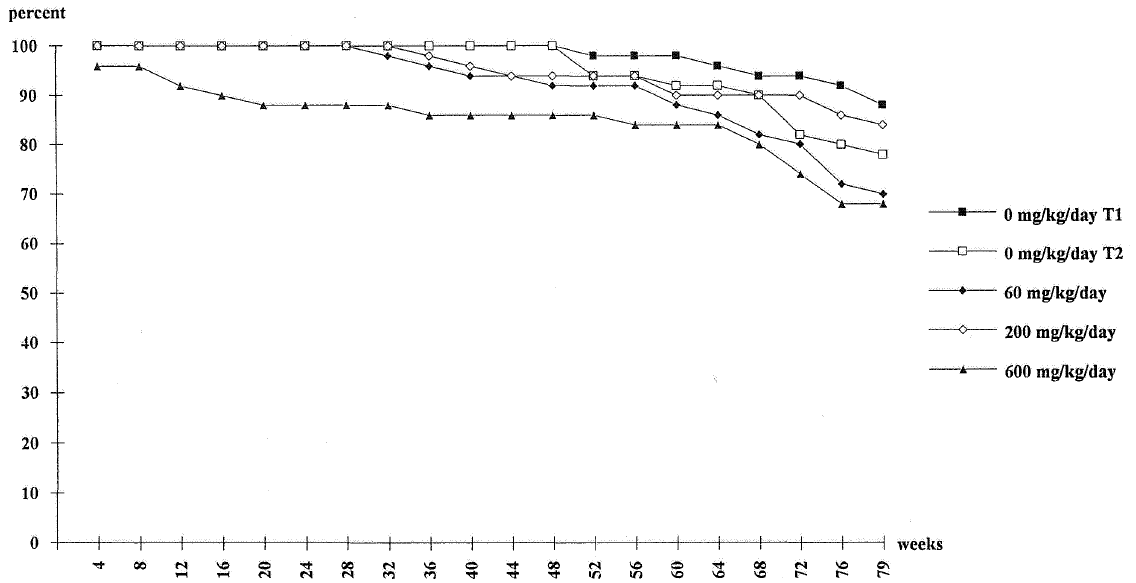
There were no clear drug-related effects on survival (Table IV.D.1.1, Figure IV.D.1.1). Higher mortality rates in LD and HD males were not attributed to drug by the sponsor because of the lack of dose-dependence and failure to observe any drug-related findings in decedents; however, it appears that there was an early drug effect at the HD (FDA statistical analysis found SS increase in mortality in HD males compared to C). The rate of mortality was similar among groups for females. There were no drug-related clinical signs or palpable masses.

Table IV.D.1.1. Mortality in mouse carcinogenicity study

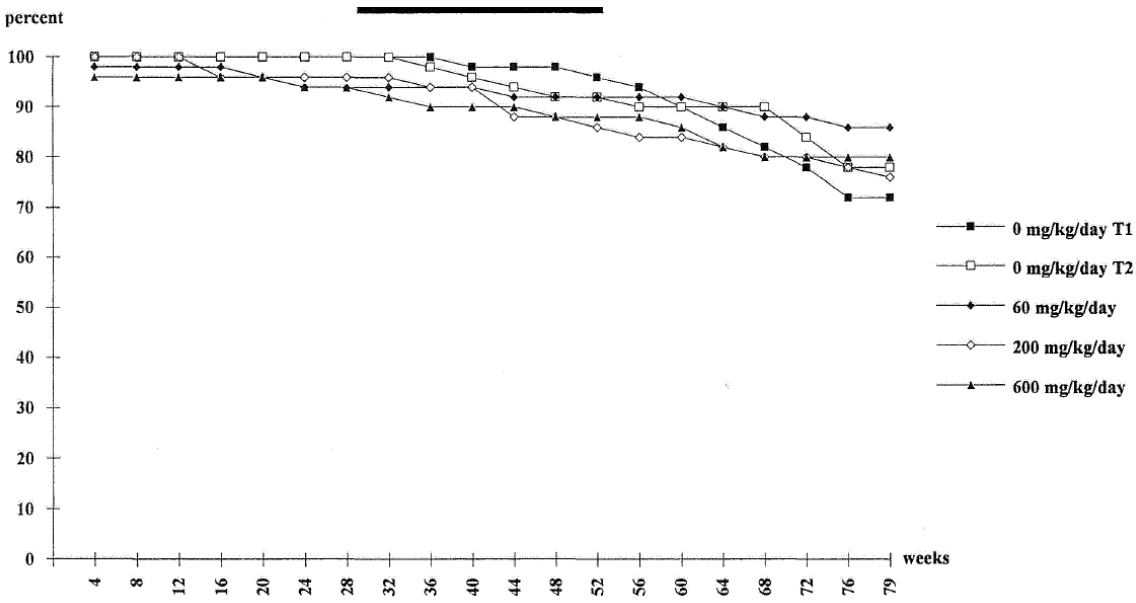
Groups Doses (mg/kg/day)	T1 0	T2 0	A 60	B 200	C 600
Number of animals/group	50	50	50	50	50
Males					
prematurely sacrificed	2	6	3	6	7
found dead	4	5	12	2	9
total	6	11	15	8	16
accidental	0	0	1	1	1
% mortality	12	22	30	16	32
% survival	88	78	70	84	68
Females					
prematurely sacrificed	8	10	3	5	4
found dead	6	1	4	7	7
total	14	11	7	12	12
accidental	1	0	0	1	2
% mortality	28	22	14	24	24
% survival	72	78	86	76	76

Figure IV.D.1.1. Survival in mouse carcinogenicity study of STP

Males



Females



ii. Body Weight

Decreased body weight gain (25 and 10% compared to T1 [SS] and T2) was observed from week 38 until the end of the treatment period in HD males. This resulted in a decrease in final BW (7% compared to T1; SS) and was considered drug-related (Table IV.D.1.2). In HD females, increased BW gain was noted from weeks 1 to 26, but at the end of the treatment period, BWs were similar among groups.

Table IV.D.1.2. Body weight in mouse carcinogenicity study of STP

Sex: Male

Dose (mg/kg/d)		0	60	200	600	
Week		T1				
78	M (3)	43	42	43	40	*
	SD	3.8	4.2	5.3	3.1	
	n (B)	45	36	43	34	

Sex: Female

Dose (mg/kg/d)		0	60	200	600
Week		T1			
78	M (1)	35	35	35	34
	SD	3.1	4.2	4.2	3.1
	n	36	43	39	39

Significance of the difference between treated and control groups
* P<0.05
** P<0.01
(1) : Dunnett test
(2) : Mann-Whitney test
(3) : Dunn test

Sample distribution-relative tests
(B) Bartlett test P<0.01
(F) Fisher test P<0.01
(K) Kolmogorov-Smirnov test P<0.01
(L) Logarithmic transformation
- Statistics excluded group

iii. Hematology

There were no drug-related effects on hematological parameters during weeks 52 or 78.

iv. Necropsy

Macroscopic -

There were no drug-related non-neoplastic macroscopic changes.

An increase in the number of animals bearing liver masses was seen at the MD and HD. According to the report, this incidence of liver masses was much higher than that usually noted in mice of this strain at the laboratory and was considered to be related to treatment.

This correlated with the higher incidences of hepatic adenomas and carcinomas seen in the treated animals microscopically.

Microscopic (no signed pathology report) –

Incidences of hepatocellular adenomas and carcinomas were markedly higher in MD and HD mice of both sexes compared to concurrent controls or spontaneous incidences of these tumors from either historical data or the literature (Table IV.D.1.3). This was considered to be a drug-related but not unexpected finding in mice treated with an enzyme inducer. The FDA statistical reviewer's analysis showed SS positive dose-response relationships for the tumor incidence rates of adenoma, carcinoma, and combined adenoma and carcinoma in livers of female mice (see statistical review by Hepei Chen). SS was not reached in males.

Table IV.D.1.3. Incidences of hepatocellular adenomas and carcinomas in mice

Doses (mg/kg/day)	0				60		200		600	
	T1		T2							
	M	F	M	F	M	F	M	F	M	F
Adenomas	4/50	-	3/50	-	4/49	-	11/50	3/50	9/50	6/50
	8%	-	6%	-	8%	-	22%	6%	18%	12%
Hepatocellular carcinoma	1/50	1/49	3/50	1/50	1/49	-	6/50	-	6/50	15/50
	2%	2%	6%	2%	2%	-	12%	-	12%	30%

Drug-related non-neoplastic microscopic findings consisted of increased incidences of liver changes at the MD and HD. Hypertrophy of the hepatocytes was noted in 13/50 (26%) males and 12/50 (24%) females at the MD and 13/50 (26%) males and 29/50 (58%) females at the HD. Intracytoplasmic concentric inclusions were noted in 6/50 (12%) of MD males and 1/50 (2%) HD females compared to none in the controls. The incidence of altered cell foci (eosinophilic, basophilic and vacuolated) and of hyperplastic nodules, was also generally higher in treated groups (Table IV.D.1.4).

Table IV.D.1.4. Incidences of altered cell foci and hyperplastic nodules

Doses	0				60		200		600	
	T1		T2							
Type of lesions	M	F	M	F	M	F	M	F	M	F
Altered cell foci acidophilic	1/50	-	-	-	-	-	-	-	-	2/50
	2%	-	-	-	-	-	-	-	-	4%
Altered cell foci basophilic	3/50	1/49	1/50	-	1/49	-	-	-	1/50	3/50
	6%	2%	2%	-	2%	-	-	-	2%	6%
Altered cell foci vacuolated	-	-	-	-	1/49	-	1/50	-	-	2/50
	-	-	-	-	2%	-	2%	-	-	4%
Hyperplastic nodules	2/50		1/50		4/49		2/50	2/50	6/50	6/50
	4%		2%		8.2%		4%	4%	12%	12%

v. Toxicokinetics

Plasma levels of STP measured 1 hour after dosing during weeks 2 and 78 are shown in Table IV.D.1.5. Other TK parameters were not determined.

Table IV.D.1.5. Plasma STP levels (µg/mL)

Groups/Sex	Doses (mg/kg/day)	Week 2	Week 78
A - Male	60	19.4 ± 2.5	19.6 ± 3.1
A - Female	60	20.8 ± 2.2	14.3 ± 2.6
B - Male	200	42.2 ± 4.5	31.9 ± 3.4
B - Female	200	58.4 ± 4.2	40.4 ± 4.1
C - Male	600	46.8 ± 4.9	41.1 ± 6.1
C - Female	600	70.9 ± 4.9	59.6 ± 4.6

c. Conclusions

Oral (gavage) administration of STP to mice for 78 weeks at doses of 0, 0, 60, 200, or 600 mg/kg/day produced no clinical signs or clear effect on survival and only a slight decrease in body weight in HD males. An increase in the number of animals bearing liver masses was noted at the MD and HD, which correlated with increased incidences of liver adenomas and carcinomas as well as increased incidences of hepatocellular hypertrophy and preneoplastic liver changes. The no-effect dose for tumorigenic effects in this study was 60 mg/kg/day, which was associated with a peak (1 hour) STP plasma level of approximately 20 µg/mL.

2. Potential tumorigenic effects in long-term administration (gavage) to rats for 104 weeks (Study no.: BC.233/GB, [redacted](b) (4) report dated 4/15/94, GLP)

a. Methods

STP (0 [0.5% CMC vehicle; T1], 0 [0.5% CMC vehicle; T2], 80, 220, or 800 mg/kg; lot # 106) was administered daily by oral gavage (10 mL/kg) for 102 weeks (the study was terminated after 102 weeks when survival rate in MD females reached ~25%) to Sprague-Dawley (Crl:CD(SD) BR) rats (50/sex/grp + 5/sex/grp TK). Observations included clinical signs, mortality, palpation for masses, food consumption and body weight, hematology (weeks 52, 78, and 101), and macroscopic and microscopic pathology examinations (full panel of tissues in C (T1) and HD and animals that died or were sacrificed prematurely; masses, lesions, and liver in all drug groups, including T2). Blood samples were taken prior to and 1, 2, 4, and 6 hrs after dosing at 102 weeks for plasma drug level measurements.

Dose selection was said to be based on the results of a 13-week oral toxicity study in rats performed at the same lab ([redacted](b) (4) but the study report was not submitted and no details were provided. (Carcinogenicity study protocol was not reviewed by the Exec-CAC.)

b. Results

i. Mortality and Clinical Observations

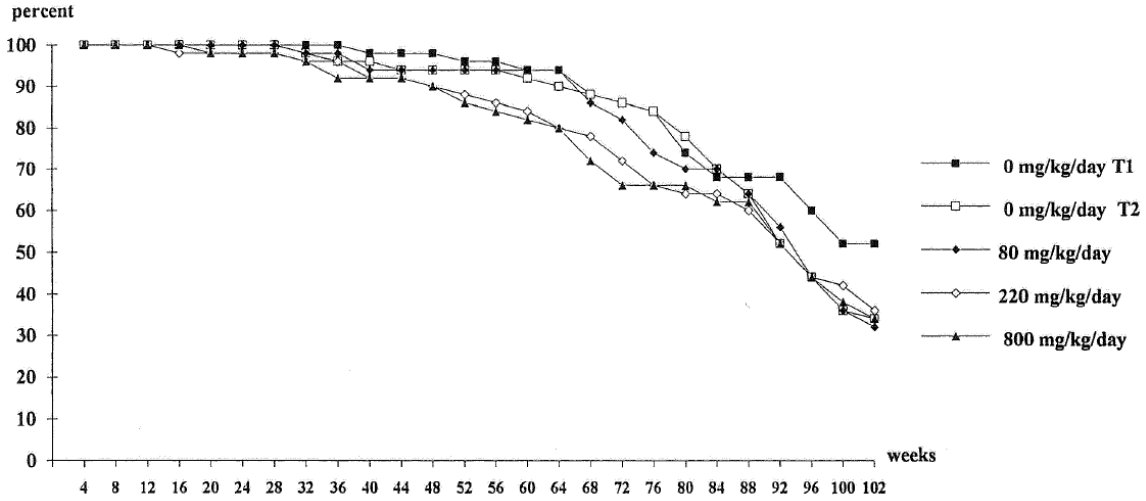
A trend toward a higher rate of mortality was seen up until week 80 in MD and HD males and females; however, at the end of the treatment period, overall survival was similar among groups, with the exception of the T1 control group males (Table IV.D.2.1, Figure IV.D.2.1). No statistically significant (SS) effects on mortality were found by the FDA statistical reviewer. Among those animals found dead or sacrificed prematurely, there were no consistent clinical, macroscopic, or microscopic findings to suggest a drug effect. The only clinical sign attributed to drug was ptyalism, which occurred with a dose-related incidence in all treatment groups.

Table IV.D.2.1. Mortality in rat carcinogenicity study

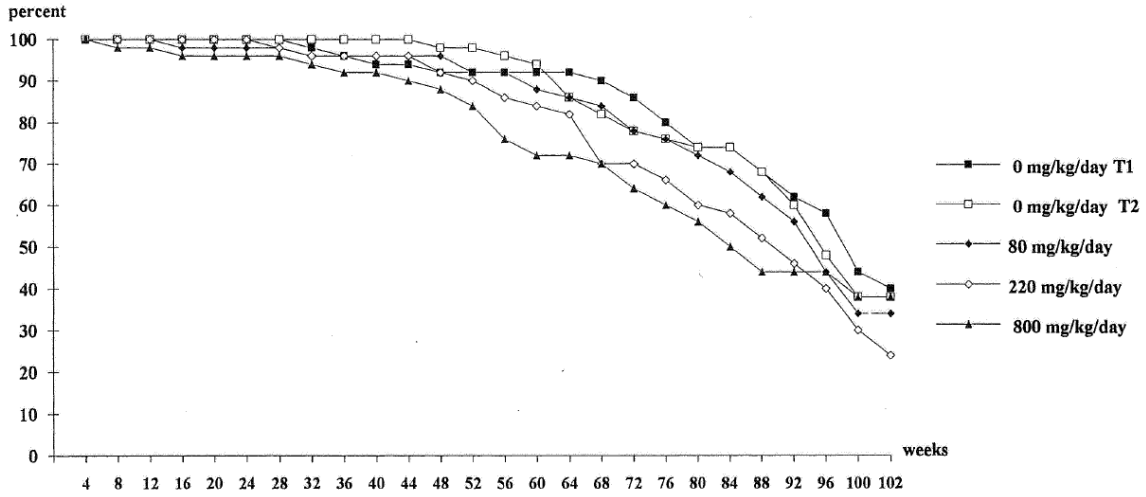
	Males					Females				
Doses (mg/kg/day)	0 T1	0 T2	80	220	800	0 T1	0 T2	80	220	800
Week 24	0	0	0	1	1	0	0	1	0	2
Week 52	2	3	3	6	7	4	1	4	5	8
Week 80	13	11	15	18	17	13	13	14	20	22
Week 102	24	33	34	32	33	30	31	34	38	31
% mortality	40	66	68	64	66	60	62	68	76	62
% survival	60	34	32	36	34	40	38	32	24	38

Figure IV.D.2.1. Survival in rat carcinogenicity study of STP

Males



Females



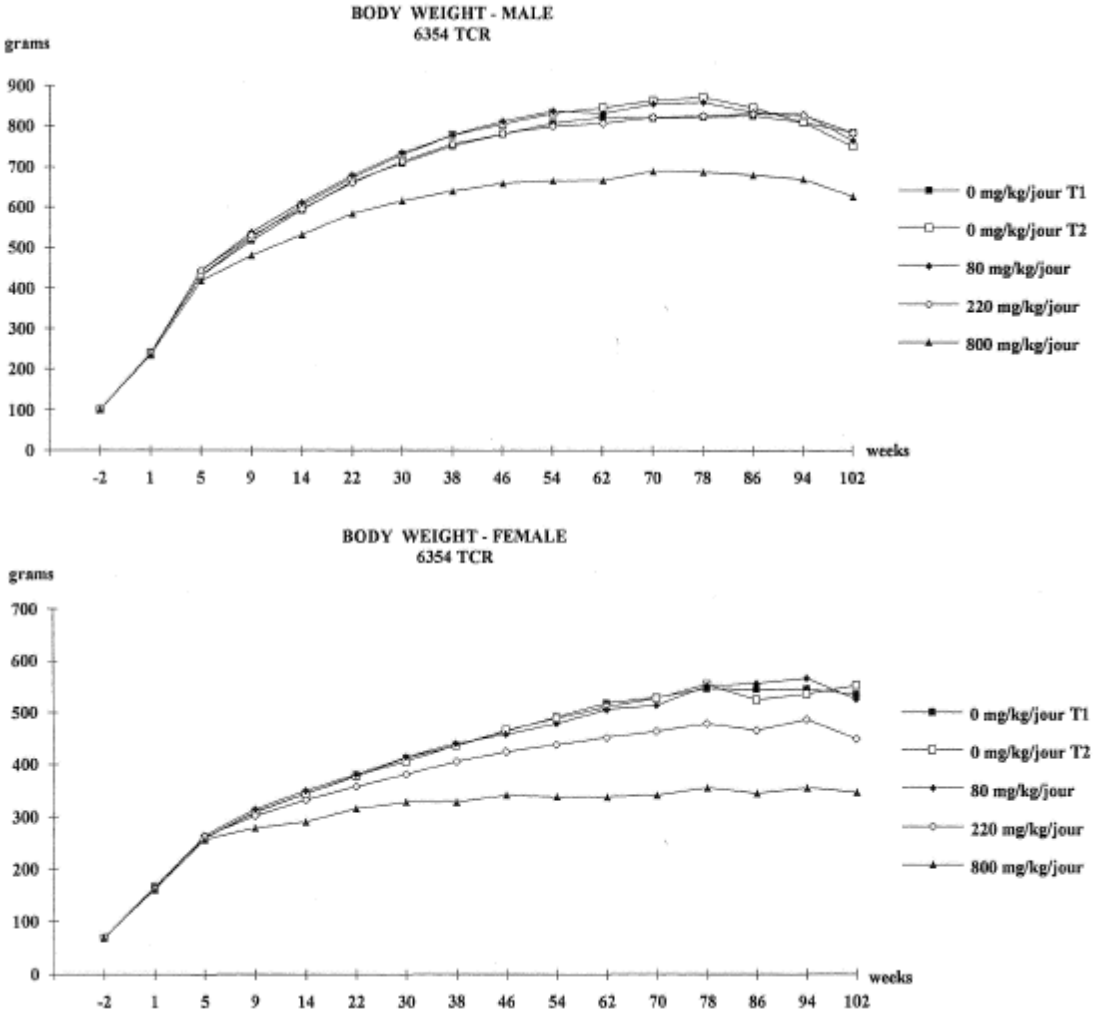
ii. Body Weight

BW gain was decreased in MD females and HD males and females, resulting in final BWs that were 16, 20, and 35% below C, respectively (SS; Table IV.D.2.2, Figure IV.D.2.2). Food consumption was actually increased (SS compared to C) in MD males and HD males and females, while efficiency of food utilization (grams of food *I* unit of body weight gain; only calculated during first 13 weeks) was decreased at the MD and HD in both sexes.

Table IV.D.2.2. Body weight in rat carcinogenicity study of STP

	Males					Females				
Doses (mg/kg/day)	0 T1	0 T2	80	220	800	0 T1	0 T2	80	220	800
Day 1 (g)	238	240	240	239	235	166	165	167	164	161
Week 5 (g)	430	432	443	442	418	261	260	265	263	257
Week 102 (g)	785	751	776	784	627	534	552	525	448	346
Overall gain (g)	547	511	536	545	392	368	387	358	284	185
% of Day 1	+230	+213	+223	+228	+67	+222	+235	+214	+173	+115

Figure IV.D.2.2. Body weight in rat carcinogenicity study of STP



iii. Hematology

There were no drug-related effects on hematological (differential white cell count only in C and HD) parameters during Weeks 52, 78, and 101.

iv. Necropsy

Macroscopic -

The number of animals showing liver enlargement was increased in MD males and HD males and females. There were no group differences in the incidence, size, or multiplicity of masses.

Microscopic (no signed pathology report) -

The number of animals with neoplasms, the number of animals with more than one primary neoplasm, and the number of benign and malignant tumors were similar among groups, indicating no effect of treatment. There was also no drug effect on tumor latencies. The FDA statistical analysis did not show any positive trend in tumor incidence among treated groups, although there were some SS differences from C (T1) at the LD and MD (Table IV.D.2.3 from statistical review by Hepei Chen). It is possible that the marked BW effects at the HD altered the dose-response by reducing tumor incidences in that group, but this seems unlikely for these tumor types.

Table IV.D.2.3. Tumor types with p-values ≤ 0.05 for pairwise comparisons of treated groups and vehicle control group in rats

Organ name	Tumor name	0 mg Vehicle (T1) P - Trend	80 mg Low (A) P – L vs. T1	220 mg Mid (B) P - M vs. T1	800 mg High (C) P - H vs. T1
Male					
Pituitary Gland	Mixed Cell Adenoma	8/38 (50) 0.6781	16/30 (40) 0.0059 $	7/19 (35) 0.1689	9/32 (50) 0.3409
Skin	Fibroma	1/35 (48) 0.7469	0/27 (43) 1.0000	6/24 (41) 0.0148 @	0/30 (50) 1.0000
	Lipoma	0/35 (48) 0.4623	1/27 (43) 0.4355	4/23 (41) 0.0209 $	1/30 (50) 0.4615
Testes	Interstitial Cell Adenoma	0/36 (50) 0.1156	1/21 (36) 0.3684	3/17 (35) 0.0290 $	3/31 (50) 0.0938
Female					
Skin	Malignant Fibrous Histiocytoma	0/34 (49) 0.4556	1/21 (38) 0.3818	4/22 (41) 0.0199 $	1/27 (50) 0.4426

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant at 0.025 and 0.05 level in rare tumor, or at 0.005 and 0.01 level in common tumor for tests of dose response relationship and pairwise comparison, respectively;
@ = Not statistically significant at 0.025 and 0.05 level in rare tumor, or at 0.005 and 0.01 level in common tumor for tests of dose response relationship and pairwise comparison, respectively;
NC = Not calculable.

Drug-related non-neoplastic microscopic findings consisted of marked increases in incidences of centrilobular hepatic cell hypertrophy and intracytoplasmic concentric inclusions, in both sexes at the MD and HD (Table IV.D.2.3). In addition, incidences of

steatosis and multifocal hepatocellular necrosis appeared to be increased somewhat in treated groups. The drug-related renal histopathology observed in the 6-month general toxicity study in rats was not as pronounced in this study, presumably due to the increased background incidences of nephrosis, particularly in males. However, chronic interstitial tubular nephrosis (in females) and contracted glomerular tuft appeared to be dose-dependently increased, as seen in the 6-month study. In addition, Leydig cell hyperplasia was increased in HD males.

Table IV.D.2.4. Non-neoplastic histopathology findings

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
IN MG/KG/DAY: T=0; A=80; B=220; C=800

DOSE GROUP:		T1		A		B		C	
	SEX:	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO.ANIMALS:	50	50	50	50	50	50	50	50
LIVER	NO. EXAM.:	50	48	48	50	50	50	50	50
- CENTRO.HEP.C.HYPERT.						15	15	40	35
- MONONUCL.CEL.AGGREG.		1		3		1			
- MICROGRANULOMA								1	
- TELANGIECTASIS.			1		2				
- STEATOSIS.		17	19	20	20	22	20	24	28
- PERIVASCULITIS									1
- PERICHOLANGITIS		18	7	1	1	3	3	9	2
- BILE DUCT PROLIFER.		13	13	3	4	4	2	8	6
- CHOLANGIOFIBROSIS.		5	2	2		1			
- INTRACYT.CONC.INCLU.								13	7
- ALTER.C.FOC.ACIDO.C.		20	23	9	10	17	7	20	21
- ALTER.C.FOC.VAC.CEL.		2				2		3	
- NODULAR HYPERPLASIA.		4	10	3	5	1	1	9	7
- AREA HEP.CEL.NECROS.					1	1			
- PELIOSIS			1	1	3	1			
- SINUSOIDAL ECTASIA		5	6	4	3	9	5	2	4
- INTERSTITIAL FIBROS.		1						1	2
- EXTRAMED.HAEMATOPOI.		4	5		3			4	
- HEPATOCELLULAR CYSTS		20	1	8		12		10	2
- BILIARY CYSTS.		16	2	3	5	1	2	1	2
- CHRONIC PERITONITIS		1							
- FOCAL TENSION LIPID.		11	11	4	9	11	6	8	3
- MULTIF.HEP.CEL.NECR.		4	3	7	6	3	9	5	7
- FOCAL HEP.CEL.NECR.			2		2		1	2	1
KIDNEYS	NO. EXAM.:	49	50	43	42	40	41	50	50
- DILATED PELVIS		6	1	4	3	8	3	2	
- EOSINOPHILIC CAST(S)		33	36	33	32	19	31	26	37
- CHR.TUB.INTERS.NEPH.		22	13	22	17	18	18	13	21
- GLOMERULONEPHROPATHY		7	11	13	11	6	6	11	9
- TUBULAR BASOPHILIA		13	10	3	1		3	15	5
- TUBULAR DILATATION		4	3	2	1		3	8	4
- INTERST.MON.CEL.AGG.		5	5	1	2		3	11	3
- TUBULAR LIPOFUSCINO.		8	7	2	7	3	9	3	14
- TUBUL.CYST DILATAT.		4	5	14	15	11	16	4	12
- POLYCYSTIC KIDNEY.						1			
- ACID.GLOB.CORT.T.EP.		2					1		
- PERIGLOMERULAR FIBR.		5						1	1
- PERITUBULAR FIBROSIS		9		2	1			7	
- CONTRACTED GLOM.TUF.					1	1	2	3	5
- GLOMERULAR HYALINOS.		1							
- MINERALIZATION			5				1	3	
- URINAR.GRAVEL, PELV.			8						
- ACUT.INFLAM.,PAPILLA						2			
- PAPILLARY NECROSIS.			2			2			
- PELV.EPIT.CEL.HYPER.			3			1			
- CHRONIC PYELITIS.		3	1				1	3	
- SUBACUTE PYELITIS.			1	1					
- ACUTE PYELITIS.			3						
- RENAL CYST.		2		2		2		3	
- LIPOMATOSIS.		1							

TESTES	NO. EXAM.:	50	36	35	50
- DEGEN.OF SEMIN.TUBU.		5	6	6	7
- INHIBIT.SPERMATOGEN.		5	6	7	7
- INTERSTITIAL OEDEMA		1			
- CALCIFICATION		3			
- POLYARTERITIS NODOSA		2	3	1	
- HYPERTROPH.SEM.TUBU.		2			
- INTERST.LYD.CEL.HYP.					8

v. Toxicokinetics

Plasma levels of STP at week 102 are shown in Table IV.D.2.5. Other TK parameters were not determined.

Table IV.D.2.5. Plasma STP levels

Groups/ Sex	Doses (mg/kg/day)	Before dosing	1 h	2 h	4 h	6 h
A - Male	80	0 -	1.63 ± 0.39	1.01 ± 0.25	0.50 ± 0.11	0.48 ± 0.08
A - Female	80	0.04 ± 0.04	3.36 ± 1.15	2.71 ± 0.67	1.58 ± 0.45	0.93 ± 0.22
B - Male	220	0.82 ± 0.53	5.74 ± 1.61	7.58 ± 2.30	6.41 ± 2.63	5.89 ± 2.62
B - Female	220	0.79 ± 0.39	6.04 ± 1.61	7.40 ± 1.86	5.93 ± 1.63	4.11 ± 1.25
C - Male	800	10.50 ± 8.18	24.98 ± 10.81	26.49 ± 10.18	35.24 ± 10.29	34.75 ± 9.60
C - Female	800	12.32 ± 7.07	27.76 ± 5.73	30.88 ± 7.63	27.30 ± 5.00	29.86 ± 4.29

d. Conclusions

Oral (gavage) administration of STP to rats for 102 weeks at doses of 0, 0, 80, 220, or 800 mg/kg/day resulted in decreased body weights (≥ 20% at HD) and increased incidences of non-neoplastic adaptive liver changes (hepatocellular hypertrophy and intracytoplasmic concentric inclusions) at the MD and HD but did not produce any clear evidence of carcinogenicity. The highest dose tested in the rat study was associated with peak plasma STP levels of ~30-35 µg/mL.

E. Reproductive and developmental toxicology

1. Stiripentol (D 306): Reproductive function and fertility study by oral route in rats (Biocodex Report BC.184/GB; dated 2/28/91; (b) (4) GLP)

a. Methods

STP (lot# 101) was administered orally (by gavage) to male and female Sprague-Dawley rats at the doses of 0 (vehicle: 1% CMC), 50, 200, or 800 mg/kg/day to males for 71 days prior to mating, during the mating period until sacrifice and to females for 15 days prior to mating, during mating, pregnancy, and lactation periods. Animals were observed for clinical signs, mortality, body weight, and food consumption. On GD 20, half of the dams were sacrificed, the fetuses delivered by C-section, and litter parameters determined. Live fetuses were weighed, sexed, and examined for external (all), skeletal (half), and visceral (half) abnormalities. The remaining dams were allowed to litter and rear their offspring. Between birth and weaning, pups were examined for body weight, viability, and development. At weaning, pups were selected, and reared to adulthood when they were mated. F1 pregnant females were allowed to litter and rear their offspring. Between birth and weaning, F2 pups were examined for body weight, viability, and development. At weaning, F1 parents and F2 pups were sacrificed. The reproductive organs of FO and F1 animals suspected of infertility were examined microscopically. No basis for dose selection was provided.

b. Results

i. Mortality and Clinical Observations

Possible drug-related deaths consisted of 1 HD male (found dead after treatment on Day 92) and 2 HD females (found dead on GD 3 and PND 8). Transient piloerection was observed in 2 HD females, but there were no other clinical signs in either sex.

ii. Body Weight

A decrease in bodyweight gain was seen in HD males (7% over dosing Days 1-99; SS) and there was a slight decrease in BW gain in females from all treatment groups during gestation (4-5% over GD 1-20, NS).

iii. Male and female fertility and reproductive indices

The fertility index was decreased by a similar amount in LD and HD groups, but no effect was seen at the MD (Table IV.E1.1).

Table IV.E.1.1. Reproductive parameters

Dose (mg/kg/day)		0	50	200	800
Paired animals					
males	N	24	23	23	24
females	N	24	24	23	24
Mated females (a)	N	23	23	22	24
Mating index	%	95.8	95.8	95.7	100.0
Pregnant females	N	21	18	20	19 (b)
Fertility index	%	91.3	78.3	90.9	82.6

(a):evidence of mating(presence of spermatozoa in the vaginal lavage) and no evidence of mating but presence of implantation sites

(b):excluding one female dead on D3 of pregnancy(implantation sites not observable)
Fertility index calculated as 19/23

iv. C-section parameters

Post-implantation loss was increased (10 vs 2.5%; SS) and the number of live fetuses decreased at the MD (90 vs 97.5%; SS), but this effect was not dose-related (Table IV.E.1.2). Fetal body weight was slightly higher in the HD group (3.91 vs 3.61 g; SS).

There were no drug-related group differences in incidences of fetal external, skeletal, or visceral abnormalities (malformations and variations).

Table IV.E.1.2. C-section data

Dose (mg/kg/day)		0	50	200	800
Pregnant	N	12	11	9	9
Dams with all Resorptions	N	0	0	0	0
Dams with Viable Fetuses	N	12	11	9	9
Corpora Lutea	TOTAL	230	198	172	164
No. per animal	MEAN	19.2 d	18.0	19.1	18.2
	S.D.	4.0	1.6	2.0	3.4
Implantation Sites	TOTAL	199	192	160	157
No. per animal	MEAN	16.6 d	17.5	17.8	17.4
	S.D.	2.7	1.0	1.0	3.0
Preimplantation Loss	TOTAL	31 f	6#	12*	7#
	%	15.6	3.1	7.5	4.5
% per animal	MEAN%	12.0 u	2.7**	6.4	3.9*
	S.D.	11.6	5.8	6.9	7.7
Fetuses	N	194	187	144	155
No. per animal	MEAN	16.2 d	17.0	16.0	17.2
	S.D.	2.8	1.1	3.2	3.3
Alive	%	100.0	100.0	100.0	100.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	N	194 f	187	144**	155
	%	97.5	97.4	90.0	98.7
No. per animal	MEAN	16.2 d	17.0	16.0	17.2
	S.D.	2.8	1.1	3.2	3.3
	MEAN%	97.4 u	97.4	89.8	98.4
	S.D.	4.0	3.9	16.6	3.3
Dead Fetuses	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
No. per animal	MEAN				
	S.D.				
	MEAN%	0.0 u	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0

Resorptions: early+late	N	5 f	5	16**	2
	%	2.5	2.6	10.0	1.3
No. per animal	MEAN	0.4 d	0.5	1.8	0.2
	S.D.	0.7	0.7	2.8	0.4
% of impl. per animal	MEAN%	2.6 u	2.6	10.2	1.6
	S.D.	4.0	3.9	16.6	3.3
Resorptions: early	N	5 f	5	15**	1
	%	2.5	2.6	9.4	0.6
No. per animal	MEAN	0.4 d	0.5	1.7	0.1
	S.D.	0.7	0.7	2.9	0.3
% of impl. per animal	MEAN%	2.6 u	2.6	9.6	0.9
	S.D.	4.0	3.9	16.9	2.6
Resorptions: late	N	0 f	0	1	1
	%	0.0	0.0	0.6	0.6
No. per animal	MEAN	0.0 d	0.0	0.1	0.1
	S.D.	0.0	0.0	0.3	0.3
% of impl. per animal	MEAN%	0.0 u	0.0	0.6	0.8
	S.D.	0.0	0.0	1.9	2.4
Postimplantation Loss	TOTAL	5 f	5	16**	2
	%	2.5	2.6	10.0	1.3
No. per animal	MEAN	0.4 d	0.5	1.8	0.2
	S.D.	0.7	0.7	2.8	0.4
% impl. per animal	MEAN%	2.6 u	2.6	10.2	1.6
	S.D.	4.0	3.9	16.6	3.3
Viable Male Fetuses	N	91 f	92	78	77
	%	46.9	49.2	54.2	49.7
Female Fetuses	N	104 f	95	66	78
	%	53.6	50.8	45.8	50.3
Fetal Body Weight (g)	MEAN	3.61 d	3.74	3.59	3.91*
	S.D.	0.28	0.28	0.20	0.22
Male Fetuses	MEAN	3.74 d	3.87	3.65	4.02*
	S.D.	0.27	0.34	0.15	0.18
Female Fetuses	MEAN	3.52 d	3.61	3.54	3.79
	S.D.	0.31	0.22	0.24	0.26

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test u=Kruskal-Wallis + Mann-Whitney U * = p<0.05 ** = p<0.01

v. Litter data and offspring development

Duration of gestation was slightly (NS) increased in MD and HD dams, and pup viability after birth was decreased in HD litters (lactation index 82 vs 99%, SS; Table IV.E.1.3). There were no other effects on delivery or litter data, including offspring bodyweights and preweaning physical (pinna unfolding, hair growth, tooth eruption, eye opening, auditory canal opening) and functional (surface righting, cliff avoidance, air righting, pupillary reflex, auditory startle) development.

Table IV.E.1.3. Litter data

SUMMARY OF POSTNATAL LOSS

Dose (mg/kg/day)		0	50	200	800
Females with Liveborn	N	8 f	7	10	10
Females Surviving Delivery	N	8 f	7	10	10
Duration of Gestation	MEAN	21.5 d	21.4	21.8	21.8
	S.D.	0.5	0.5	0.4	0.4
with Stillborn Pups	N	1 f	1	2	0
	%	13	14	20	0.0
with all Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
with Entire Liveborn Litter Dying and/or Missing, Cannibalized, Culled					
days 0-4	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 5-21	N	0 f	0	0	2
	%	0.0	0.0	0.0	20
days 0-21	N	0 f	0	0	2
	%	0.0	0.0	0.0	20

Litters with Liveborn Pups	N	8	7	10	10
Pups Delivered (total)	N	140	114	157	148
	MEAN	17.5 d	16.3	15.7	14.8
	S.D.	2.3	2.4	2.4	3.9
Liveborn	N	139 f	113	155	148
Live Birth Index	%	99	99	99	100
Stillborn	N	1 f	1	2	0
	%	0.7	0.9	1.3	0.0
Uncertain	N	0	0	0	0
Culled (total)	N	0	0	0	17
Cannibalized	N	1	1	0	13
Missing	N	0	0	0	0
Died	N	2	1	9	10
Liveborn, not culled prior to day 21	N	139	113	155	131
Pups Dying, Missing, and/or Cannibalized					
day 0	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 1-4	N	2 f	1	4	3
	%	1.4	0.9	2.6	2.0
days 5-7	N	0 f	1	3	13#
	%	0.0	0.9	1.9	8.8
days 8-14	N	1 f	0	2	6
	%	0.7	0.0	1.3	4.1
days 15-21	N	0 f	0	0	1
	%	0.0	0.0	0.0	0.7
Pups Surviving 4 days	N	137 f	112	151	145
Viability Index	%	99	99	97	98
Pups Surviving 21 days	N	136 f	111	146	108#
Lactation Index	%	99	100	99	82
Implantation Sites	N	142	121	162	156
per Litter	MEAN	17.8 d	17.3	16.2	15.6
	S.D.	2.4	2.7	2.1	3.6

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test # = p<0.001

vi. F1 mating, fertility, and litter viability

The mating and fertility indices and litter viability for mated F1 animals were similar among groups.

c. Conclusions

Oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to male and female Sprague-Dawley rats (24/sex/group) prior and during mating and throughout pregnancy and lactation in females did not result in any clear effects on fertility or reproductive performance. The HD induced some maternal/paternal toxicity (clinical signs in females, mortality in 1 male and 2 females, reduced body weight gain of males and females). The only developmental toxicity noted was decreased viability of pups during lactation at the HD. No signs of toxicity and no effects on fertility were noted in F1 animals and the development of F2 pups was not affected.

2. Expert evaluation to determine any possible teratogenic action of the D.306 in the mouse (Biocodex Report BC.105/GB-1; dated 5/28/74; [redacted] non-GLP)

a. Methods

Mated female CD mice (25/group) received oral (gavage, 1 mL/20 g) doses of 0 (0.5% gum arabic vehicle), 50, 200, or 800 mg/kg/day STP (batch # 28) from GDs 6 through 16. Maternal BW was measured throughout gestation. Animals were killed on GD 19 and pregnancy outcome was evaluated. Fetuses were only evaluated for external abnormalities. No basis for dose selection was provided.

b. Results

i. Maternal observations

There were no drug-related maternal deaths or clinical signs and no effects on maternal BW gain (Table IV.E.2.1).

Table IV.E.2.1. Maternal and fetal body weight in grams (groups correspond to C, LD, MD, and HD)

Group	Day	DT	DL	DM	DF
Mean weight of groups during the course of gestation	1	36,68	32,76	34,44	33,32
	4	40,04	35,28	37,88	36,24
	7	41,76	36,24	37,32	38,16
	10	43,40	38,52	38,72	39,44
	14	48,56	43,76	43,08	44,80
	19	55,84	53,58	55,48	55,83
Weight gain from 1st to 19th day		19,16	20,92	21,04	22,56
Mean weight of foetuses on 19th day		1,44	1,32	1,35	1,28
Mean weight of placentas on 19th day		0,11	0,10	0,10	0,11

ii. Pregnancy and fetal evaluations

Fetal BW was reduced at all doses (Table IV.E.2.1). Resorptions were dose-dependently increased, but there was no corresponding decrease in number of fetuses/litter, so this could in part be attributed to the higher numbers of implantations in treatment groups (Table IV.E.2.2).

Table IV.E.2.2. Caesarean-section observations

Group	DT	DL	DM	DF
Mated females	25	25	25	25
Females with resorption of whole litter	0	0	0	0
Number of females alive on 19th day	25	25	25	25
Gestations continuing until 19th day	21	22	19	20
Number of live foetuses (L)	205	220	191	215
Mean number of live foetuses per litter	9,8	10,0	10,1	10,8
Number of dead foetuses (D)	10	9	8	9
Total number of foetuses on 19th day (L + D)	215	229	199	224
Number of resorptions (R)	21	26	29	36
Percentage of resorptions ($\frac{R}{L}$)	10,2	11,8	15,2	16,7
Total number of implantations (L + D + R)	236	255	228	260
Mean number of implantations per litter	11,2	11,6	12,0	13,0

There was an apparent effect on embryofetal development at the HD based on the fetal incidences of external malformations: 2/206, 0/218, 2/191, and 10/215 in the C, LD, MD, and HD groups, respectively. The malformations were ectocardia in 1 C fetus, exencephaly in 1 C fetus, torsion of the tail in 1 MD fetus, and cleft palate in 1 MD fetus and 10 fetuses from 2 HD litters.

c. Conclusions

Oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg) to pregnant CD mice throughout organogenesis (GDs 6-16) had no effects on the progression of gestation nor any maternal toxicity but resulted in increased resorptions and decreased fetal BWs at all doses and an increased incidence of malformations at the HD. Cleft palate was seen in 1 MD fetus and 10 fetuses from 2 HD litters.

3. Teratogenic action of D.306 in the pregnant mouse and action in gestation (Biocodex Report BC.105/GB-2; dated 11/29/74; [redacted](b) (4) non-GLP)

a. Methods

Mated female CD mice (22/group) received oral (gavage, 1 mL/20 g) doses of 0 (0.5% gum arabic vehicle) or 800 mg/kg/day STP (batch # 28) from GDs 4 through 14. Animals were killed on GD 19 and pregnancy outcome was evaluated. Fetuses were only evaluated for external abnormalities. This study was conducted to follow up on the findings in the previous study, i.e., an increased incidence of cleft palate in mice exposed to STP.

b. Results

i. Maternal observations

There were no drug-related maternal deaths or clinical signs and no effects on maternal BW gain (Table IV.E.3.1).

Table IV.E.3.1. Maternal and fetal BW in grams (groups correspond to C and HD)

Group	Day	DT	DF
Mean weight of groups during gestation	1	34,8	34,9
	4	34,9	37,5
	7	36,9	37,8
	10	39,7	37,6
	14	45,0	46,1
	19	60,3	59,2
Weight gain from 1st to 19th d.		25,5	24,3
Mean weight of foetuses on 19th d.		1,36	1,30
Mean weight of placentas on 19th d		0,10	0,10

ii. Pregnancy and fetal evaluations

Fetal BW was reduced at the HD (Table IV.E.3.1). Numbers of dead fetuses and resorptions were increased in the drug-treated group (Table IV.E.3.2).

Table IV.E.3.2. Caesarean-section observations

Group	DT	DF
Fertilized females	22	22
Abortions	0	0
Number of females alive on 19th d.	22	22
Gestations continuing until 19th d	22	22
Mean number of live foetuses per litter	11,6	11,1
Number of resorptions	27	31
Number of live foetuses	256	244
Number of dead foetuses	1	5
Percentage of resorptions	10,5	12,7
Total number of foetuses on 19th d	257	249

The fetal and litter incidences of external malformations were increased in the drug-treated group (Table IV.E.3.3). The number of litters with malformed fetuses (8/22 vs 4/22) and with fetuses with cleft palate (4/22 vs 2/22) was doubled in the drug-treated group compared to C.

Table IV.E.3.3. Fetal abnormalities

Abnormality	DT	DF
Number of malformed foetuses	6/256	8/244
Number of litters with malformed foetuses	4/22	8/22
Cleft palate	3	4
Ocular lesions	2	0
Exencephaly	0	1
Rotation hind limbs	1	1
Hematomas	0	2
Number of litters with cleft palate	2/22	4/22

c. Conclusions

Oral (gavage) administration of STP (0 or 800 mg/kg) to pregnant CD mice during organogenesis (GDs 4-14) resulted in increased mortality, decreased BW, and an increased incidence of malformations in drug-exposed fetuses. There was no evidence of maternal toxicity at the dose tested.

4. Study of the influence of D.306 on internal and skeletal morphology of the mouse foetus (Biocodex Report BC.105/GB-3; dated 11/29/74; [redacted] non-GLP)

a. Methods

Mated female CD mice (25/group) received oral (gavage, 1 mL/20 g) doses of 0 (0.5% gum arabic vehicle), 50, 200, or 800 mg/kg/day STP (batch # 28) from GDs 6 through 16. Animals were killed on GD 19, laparotomies performed, and fetuses were evaluated for visceral (half) and skeletal (half) abnormalities only. Maternal effects and other pregnancy and fetal parameters were not assessed. No basis for dose selection was provided.

b. Results

i. Fetal evaluations

The only visceral malformation reported was a single case of encephalocele in a HD fetus. No visceral variations were noted. There were no apparent drug-related differences in incidences of skeletal malformations or variations. However, the degree of ossification in drug-treated fetuses was greater than in the controls.

c. Conclusions

Oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg) to pregnant CD mice during organogenesis (GDs 6-16) did not produce any effects on incidences of visceral or skeletal abnormalities.

5. Determination of any possible teratogenic action of D.306 in the rabbit (Biocodex Report BC.105/GB-5; dated 1/15/75; [redacted] non-GLP)

a. Methods

Mated female (wild burgundy strain) rabbits (18-28/group) received oral (gavage, 1 mL/kg) doses of 0 (5% gum arabic), 50, 200, or 800 mg/kg/day STP (batch # 28) from GDs 8 through 21. Maternal BW was measured throughout gestation. Animals were killed on GD 28, laparotomies were performed, and pregnancy outcome evaluated. Fetuses were examined for external, visceral (~1/3), and skeletal abnormalities (~2/3; methods not described).

b. Results

i. Pregnancy observations

Rates of abortion were high in all groups, but highest at the MD and HD: 38, 28, 56, and 46, at the C, LD, MD, and HD, respectively. This resulted in inadequate numbers of litters at all doses (13, 12, 10, and 7 in respective groups). Maternal BW gain was dose-dependently decreased (net loss at MD and HD; Table IV.E.5.1).

Table IV.E.5.1. Maternal and fetal body weight in grams (groups correspond to C, LD, MD, and HD)

Group	Day	DT	DL	DM	DF
Mean weight of groups during gestation	1	3005	3014	3070	3217
	8	3005	3014	3070	3217
	15	3031	3004	3086	3059
	21	3099	3072	2995	2980
	28	3096	3131	3015	2995
Weight gain from 1st to 28th d.		+ 91	+117	- 55	-222
Mean weight of foetuses on 28th d.		23,76	20,87	20,13	21,88
Mean weight of placentas on 28th d.		5,20	4,36	5,51	4,60

ii. Litter parameters and fetal evaluations

Fetal body weights were decreased at all doses, but not dose-dependently (Table IV.E.5.1). Although resorptions appeared to be increased at the MD and HD, there were no drug-related effects on numbers of live fetuses per litter Table (IV.E.5.2).

Table IV.E.5.2. Caesarean-section observations

Group	DT	DL	DM	DF
Fertilized females	21	18	27	28
Abortions	8	5	15	13
Gestations continuing to 28th day	13	12	10	7
Number of females alive on 28th day	21	17	25	19
Number of live foetuses	69	94	59	56
Mean number of live foetuses per litter	5,3	7,8	5,9	8,0
Number of dead foetuses	5	0	8	2
Total number of foetuses on 28th day	74	94	67	58
Number of resorptions	77	51	96	100
Percentage of resorptions	111,5	54,2	162,7	178,5
Total number of implantation sites	151	145	163	158
Mean number of implantation sites	7,2	8,5	6,5	7,9

There were no apparent drug-related increases in incidences of malformations or variations.

c. Conclusions

Oral (gavage) administration of STP to pregnant rabbits throughout organogenesis (GDs 8-21) at doses of 0, 50, 200, or 800 mg/kg/day resulted in drug-related developmental (increased resorption at MD and HD, decreased fetal BW at all doses) and maternal (decreased BW gain and increased abortion at the MD and HD) toxicity.

6. Study of the influence of D.306 on the peri- and postnatal development in the rat (Biocodex Report BC.105/GB-4; dated 11/29/74; [redacted](b) (4) non-GLP)

a. Methods

Female S-D rats (10/grp) were given oral (gavage) doses of STP (0 (5% gum arabic vehicle), 50, 200, or 800 mg/kg/day) from GD 6 until weaning on PND 23. Pups were counted after birth and live animals were weighed on PNDs 1, 4, 7, 14, and 21. Offspring were weaned on PND 23, separated and weighed once weekly until the age of 2 1/2 months. At that time, 5/sex/group were mated for a maximum of 1 month and reproductive outcomes evaluated. Other developmental parameters were not assessed.

b. Results

i. Maternal observations

There were no drug-related deaths, but there was a decrease (12%) in BW gain during the gestational dosing period at the HD (Table IV.E.6.1).

Table IV.E.6.1. Maternal BW gain during gestation

	DT	DL	DM	DF
First day	233.5	187.0	210.5	232.5
Sixth day	268.5	215.7	247.2	264.5
Tenth day	286.5	255.5	275.5	276.4
Thirteenth day	309.0	269.5	283.3	289.5
Sixteenth day	338.5	317.5	303.1	316.2
Twentieth day	363.3	341.1	353.3	348.1
Difference in weight the sixth and twentieth days of gestation.	94.8	125.4	106.1	83.6

ii. Litter and preweaning offspring parameters

At parturition, there were no group differences in numbers of stillbirths or live pups per litter, but pup BW was decreased at the HD (Table IV.E.6.2). During lactation, offspring viability was decreased at the HD and offspring BW gain was decreased at the MD and HD.

Table IV.E.6.2. Preweaning offspring parameters

		DT	DL	DM	DF
Number of live offspring	Birth	96	90	94	78
	4 d	95	90	94	76
	7 d	95	90	94	66
	14 d	95	90	94	66
	21 d	95	90	94	66
Mean weight	Birth	6,6	7,6	7,2	5,8
	4 d	9,8	11,8	9,7	8,8
	7 d	16,9	15,6	13,2	11,2
	14 d	27,7	28,6	25,5	22,3
	21 d	41,0	44,6	38,8	30,1
Difference in weight between first and 21st day		34,4	37,0	31,6	24,3
Number of live litters on the 21st in comparison with the number of initial litters		9/10	9/9	8/8	6/7

iii. Postweaning offspring parameters

There were no differences among groups in offspring postweaning BW gain or fertility and reproductive performance parameters.

c. Conclusions

Oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to female S-D rats from GD 6 through PND 23 resulted in maternal (decreased BW gain at the HD) and developmental toxicity (decreased pup BW at birth and increased mortality during lactation at HD, decreased BW gain during lactation at MD and HD).

7. Peri and postnatal study by oral route in rats (Biocodex Report BC.328/GB; dated 4/2/94; [redacted] (b) (4) GLP)

a. Methods

Female S-D rats (20/grp) were given oral (gavage, 5 mL/kg) doses of STP (0 (0.5% CMC vehicle), 50, 200, or 800 mg/kg/day; lot# 106) from GD 16 to PND 21. Females were observed daily for clinical signs, mortality, food consumption, and body weight and allowed to deliver and rear their offspring until PND 21. The litters were examined daily for clinical signs and the number of live, dead, or cannibalized pups and BW and physical and reflex development of the pups were assessed throughout the lactation period.

b. Results

i. Maternal observations

Two HD females were sacrificed prematurely due to drug-related poor clinical condition: 1 on GD 22 (not pregnant) and 1 on PND 1 post-partum (delivered but entire litter was dead on PND 1). Clinical signs, indicative of poor condition, consisted of piloerection, hypokinesia, round back, cold to the touch in 4/20 females, usually from PND 1 to 3 (2/4 were sacrificed prematurely). Other clinical signs at the HD were ptyalism in 1 female on PNDs 2 and 3 and reddish-soiled urogenital area in 6/20 females on PND 1 or 2. BW gain was decreased in HD females during the gestational dosing period (44 vs 66 g; SS). There were no effects on BW gain during the lactation period. The duration of gestation was similar among groups and there were no signs of difficult or prolonged parturition.

ii. Litter and preweaning offspring parameters

At parturition, the live birth index was 100% in all groups, with no stillborn pups noted, and the numbers of pups delivered were similar across groups. However, pup survival was markedly reduced at the HD, mostly between PNDs 1 and 4: HD mortality was 45% on PND 4 compared to 2.9% in C (Table IV.E.7.1). Pup BW was decreased at birth and during lactation at the HD (Table IV.E.7.2).

Physical development (pinna unfolding, hair growth, tooth eruption, eye and auditory canal opening) appeared to be unaffected by treatment. Deficits in 2 indices of reflex development were seen at the HD: the % of pups with a positive response to the surface righting and air righting reflex tests was decreased compare to C (88.2 and 84.7% vs 94.9 and 97.4%, respectively; SS).

c. Conclusions

Oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to female S-D rats from GD 16 through PND 21 resulted in maternal (mortality, clinical signs, decreased BW gain) and developmental (decreased pup survival during PNDs 1-4, decreased pup BW at birth and throughout lactation, deficits in reflex development) toxicity at the HD.

Table IV.E.7.1.

SUMMARY OF REPRODUCTIVE AND LITTER DATA

Dose (mg/kg/day)		0	50	200	800
Litters with Liveborn Pups	N	18	18	19	18
Pups Delivered (total)	N	243	217	247	231
	MEAN	13.5 d	12.1	13.0	12.8
	S.D.	2.2	3.2	3.5	3.7
Liveborn	N	243 f	217	247	231
Live Birth Index	%	100.0	100.0	100.0	100.0
Stillborn	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
Uncertain	N	0	0	0	0
Culled (total)	N	0	0	0	0
Cannibalized	N	5	1	4	47
Missing	N	0	0	0	0
Died	N	5	5	3	60
Liveborn, not culled prior to day 21	N	243	217	247	231
Pups Dying, Missing, and/or Cannibalized					
day 0	N	0 f	0	0	0
	%	0.0	0.0	0.0	0.0
days 1-4	N	7 f	1	4	104#
	%	2.9	0.5	1.6	45.0
days 5-7	N	1 f	4	3	3
	%	0.4	1.8	1.2	1.3
days 8-14	N	1 f	1	0	0
	%	0.4	0.5	0.0	0.0
days 15-21	N	1 f	0	0	0
	%	0.4	0.0	0.0	0.0
Pups Surviving 4 days	N	236 f	216	243	127#
Viability Index	%	97.1	99.5	98.4	55.0
Pups Surviving 21 days	N	233 f	211	240	124
Lactation Index	%	98.7	97.7	98.8	97.6
Implantation Sites	N	262	237	270	257
per Litter	MEAN	14.6 d	13.2	14.2	14.3
	S.D.	1.9	3.5	3.5	3.7

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test # = p<0.001

Table IV.E.7.2.

SUMMARY OF REPRODUCTIVE AND LITTER DATA

Dose (mg/kg/day)		0	50	200	800
Live Pups/Litter					
day 1	MEAN	13.4 d	12.0	12.9	10.9
	S.D.	2.1	3.2	3.4	4.7
day 4	MEAN	13.1 d	12.0	12.8	7.5#
	S.D.	2.1	3.2	3.4	6.9
day 7	MEAN	13.1 d	11.8	12.6	7.3#
	S.D.	2.1	3.1	3.3	6.8
day 14	MEAN	13.0 d	11.7	12.6	7.3#
	S.D.	2.1	3.1	3.3	6.8
day 21 preculling	MEAN	12.9 d	11.7	12.6	7.3#
	S.D.	2.0	3.1	3.3	6.8
Pup Weight/Litter (grams)					
day 1	MEAN	6.6 d	7.0	6.9	5.7**
	S.D.	0.5	0.8	0.8	0.9
day 4	MEAN	9.8 d	10.4	10.5	7.9**
	S.D.	1.0	1.6	1.7	1.4
day 7	MEAN	14.8 d	15.6	15.6	11.5**
	S.D.	1.6	2.1	2.8	2.7
day 14	MEAN	28.0 d	29.3	28.7	22.9*
	S.D.	3.0	4.3	6.2	3.1
day 21 preculling	MEAN	42.5 d	44.2	41.2	32.5**
	S.D.	5.6	7.4	9.4	4.2
Sex Ratio - Male Pups:Total Pups					
day 0	N	142 f	113	113**	100**
	%	58.4	52.3	45.7	45.9
day 21	N	136 f	109	111**	64
	%	58.4	51.7	46.3	51.6

Statistical key: d=ANOVA + Dunnett-test f=Fishers exact test * = p<0.05 ** = p<0.01 # = p<0.001

F. Other studies

Qualification of impurities

1. Toxicity of impurities from the synthesis and breakdown of D306 (Biocodex Report BC.262/EN, study conducted 9/87, 3-page report dated 1/29/96; conducted by Biocodex, Paris, France; non-GLP)

Male mice (Swiss EOPS Iffa-Credo) were administered STP (batch 13; 1250, 1500, 1750, or 2000 mg/kg) or the degradation products (b) (4) (1500 or 2000 mg/kg) and toxic signs and percent mortality were compared (only mortality data provided in report). While STP produced up to 90% mortality at the HD, neither of the impurities produced any deaths or, according to the report, showed any signs of toxicity at the doses studied. The identity of the impurities tested in this study is unclear; however, according to the FDA review chemist, there were no concerns regarding impurities (limits are NMT 0.1% per ICH guidelines) and they were not detected on stability. All excipients are present in the drug product at lower levels than the currently approved drug products per the IID list except for (b) (4) ((b) (4) MDD based on 85 kg adult). The IID limit by the oral route is (b) (4) mg.

V. SUMMARY AND EVALUATION

Pharmacology

The mechanism of the anticonvulsant action of STP remains unclear. An indirect effect through the inhibition of P450 cytochromes (CYP1A2, CYP2C9, CYP2C19, and CYP3A4) is thought to be involved in its clinical efficacy. STP was shown to increase the plasma concentrations of known anticonvulsant drugs, such as carbamazepine (CBZ), that are metabolized by these cytochromes. STP inhibited the formation of the epoxy metabolite of CBZ, which was thought to provide an additional beneficial effect, since CBZ-induced toxicity, including teratogenicity, has been attributed to the epoxide (Finnell et al., 1995). A similar metabolic interaction was seen with clobazam (CLB): STP was shown to inhibit the N-demethylation of CLB to the active metabolite N-desmethylclobazam (NCLB) mediated by CYP3A4 and CYP2C19 and to more strongly inhibit the hydroxylation of NCLB to the inactive 4'-hydroxy-N-desmethylclobazam by CYP2C19 (Tran et al., 2005). This metabolic interaction could increase the effectiveness of CLB by increasing plasma levels of CLB and NCLB in patients treated with the CLB/STP combination. However, STP also demonstrated independent anticonvulsant activity in several animal models, including the maximal electroshock (MES) and sc pentylenetetrazol (PTZ) models, with what were considered (b) (4) to be adequate protective indices (TD50/ED50). However, it should be noted that the plasma levels associated with efficacy in these models was relatively high compared to those expected clinically. Therapeutic doses of STP reportedly result in steady state plasma concentrations of 4-22 µg/mL. A Cmax of 13 µg/mL was reported in the STP-1 study in Dravet patients receiving a mean dose of 48 mg/kg (Inoue et al., 2015). Chiron et al. (2000) reported that a mean dose of 49 mg/kg/day was associated with a mean plasma concentration of 10 µg/mL.

In other studies providing evidence for intrinsic activity, STP increased GABAergic postsynaptic currents when applied to CA3 pyramidal neurons of hippocampus in immature rats at what were considered clinically relevant concentrations, was effective in a rodent model of refractory pilocarpine-induced status epilepticus, attenuated seizure activity in Nav1.1 mutant zebrafish which spontaneously exhibits abnormal electrographic activity and convulsive behavior, and was effective in preventing hyperthermia-induced seizures in young (1 month of age) but not older (5 months of age) animals in a heterozygous SCN1A mutant mouse model of Dravet syndrome that presents with hyperthermia-induced seizures. It should be pointed out that very few of these pharmacology studies of STP were carried out by the sponsor (only very early anticonvulsant testing in basic models, including one conducted (b) (4) for the sponsor, and pharmacologic interaction studies), so most of this information comes from literature reports.

Clinical evidence provided by the sponsor included the results of an observational study performed in Japan on 23 patients with Dravet syndrome, in which 48-61% of patients experienced a more than 50% decrease in seizure frequency. These rates are close to those observed in Europe, although only about 1/2 of the Japanese patients received CLB as co-medication, and 20% of them had a CYP2C19 inactive allele *2 or *3. Post hoc analysis of the pharmacokinetic data from two randomized controlled trials performed in Dravet syndrome in France indicated that responder rate was similar whether the patients exhibited an increase in CLB, NCLB, or NCLB/CLB on STP or not. For example, 78% of children experienced an increase of NCLB/CLB ratio, but their responder rate (65%) was not different from those who did not (75%). These data are thought by the sponsor to show that the antiepileptic effect of STP in Dravet syndrome does not depend only on its pharmacokinetic interaction with CLB (Chiron, Orphan Drugs: Research and Reviews 2014:4 29–38).

Mechanism of action studies have focused on GABA potentiation. Literature studies indicate that STP is a positive allosteric modulator of the GABAA receptor at a site distinct from that of other GABAA receptor modulators (STP did not block bicuculline- or picrotoxin-induced seizures in vivo). In studies conducted by the sponsor, co-administration of STP (100 mg/kg ip) and a

benzodiazepine (diazepam [0.75 mg/kg ip] or clonazepam [0.025 mg/kg ip]) was said to result in more than additive protection against metrazol-induced seizures in mice, but the data were not convincing. These results were thought to be consistent with in vitro studies showing that when STP at clinically relevant concentrations was applied to brain slices together with diazepam, a further enhancement of GABAergic neurotransmission was observed. In addition to possible allosteric modulation of the GABAA receptor, STP was recently reported (in literature) to increase GABA release through a presynaptic effect.

Formal safety pharmacology studies were not conducted. In a CV study conducted in 2 mongrel dogs, STP (2.5 and 5 mg/kg iv) decreased blood pressure and heart rate slightly at both doses tested. During the (b) (4) study, the effects of high doses of STP (600 to 1800 mg/kg ip in groups of 2 animals) were measured in male CF mice. At the 600 mg/kg dose, neurological deficit, as evidenced by inability to remain on the rotarod (the authors describe this as "ataxia"), decreased motor activity and decreased respiration with cyanosis were observed; animals recovered within 1 hour. The same symptoms were observed at the 1200 mg/kg dose, but lasted longer (2 hours). At the 1800 mg/kg dose, decreased motor activity, spasticity, "ataxia," reduced rotarod performance, sedation, ptosis, muscle relaxation, loss of righting reflex, decreased respiration with cyanosis were observed and 1 of 2 animals died.

ADME

ADME information was based on literature reports. Based on studies in rat and monkey, STP formulated in 1% carboxymethyl cellulose (CMC) or PEG 400 appeared to be rapidly absorbed after oral administration, with peak levels seen at ~1 hour. In rhesus monkey (not used in toxicity testing), the extent of oral absorption was <30%, which was attributed to poor solubility and a hepatic first pass effect. Distribution studies in Wistar and S-D rats indicated that labeled STP partitions into liver, adrenal gland, kidney, lung, placenta, brain, spinal cord, and fetus. Brain/plasma ratios in rats varied between 0.25 and 0.70 with no difference between (R)-(+) and (S)-(-) enantiomers. Chiral inversion of (R)- to (S)-STP was found following oral administration in the rat: after administration of (S)-STP, the plasma contained only that enantiomer (AUC S/R ratio = 47.7); when (R)-STP was given, both enantiomers were detected in plasma yielding an AUC S/R ratio = 0.86. Also, when racemic drug was administered, (S)-STP was predominant in plasma with an AUC S/R ratio of 3.32. However, the (R)-enantiomer was eliminated more rapidly than the (S)-enantiomer, as reflected in a higher plasma clearance (1.64 L/hIkg versus 0.557 L/hIkg) and a shorter half-life (2.83 h versus 6.50 h) when the enantiomers were administered individually to male S-D rats. The opposite pattern was reported in humans, in which the PK results indicated conversion of (S)- to (R)-STP and a higher apparent oral clearance for the (S)-enantiomer. (R)-STP was the predominant enantiomer in plasma after oral administration of the racemate (1200 or 2400 mg) to 6 healthy subjects (AUC R/S ratio of 4.1). Enantioselectivity was also observed with respect to anticonvulsant activity: (R)-STP was 2.4 times more potent than its antipode against PTZ-induced clonic convulsions in male S-D rats.

Based on literature studies in which urinary metabolites were characterized in Sprague-Dawley rats and rhesus monkeys, the metabolism of STP is thought to primarily involve glucuronidation, opening of the methylenedioxyphenyl ring, and hydroxylation. Thirteen of 15 rat urinary metabolites were found in common with human urinary metabolites. STP was also extensively metabolized in rhesus monkey and the urinary metabolite profile agreed qualitatively with the findings in rats and humans. Excretion studies showed that in rat and monkey the fraction of dose appearing unchanged in urine is less than or equal to 1%, consistent with elimination by metabolism. A multiphase elimination curve was seen in rats after iv administration of ^{3}H-STP plasma concentrations dropping rapidly in the first phase, followed by a much slower decrease during the second phase. The same multiphasic pattern of STP disappearance from plasma was observed in monkeys following iv administration of 3 different doses. Plasma clearance varied with dose in this study, indicating nonlinearity, and Michaelis-Menten type nonlinear pharmacokinetics has been demonstrated for STP in humans.

Safety Margin

While TK data were collected in the pivotal toxicity studies, AUCs were not calculated, so the usual exposure comparisons cannot be made. However, maximal plasma levels associated with the no-effect doses for renal histopathology in rats and monkeys were similar to or below those expected clinically (reportedly up to 22 µg/mL). Clinical plasma level data were also limited, but in a PK study in 12 healthy male adult subjects, a single oral dose of 2000 mg resulted in mean Cmax and $AUC_{0\text{-}inf}$ values of 13.2 µg/mL and 87.7 µg.hr/mL, respectively. In the mouse carcinogenicity, plasma levels were only determined at one time point and there was only one dose in common with the 13-week mouse toxicity study, in which samples were collected at multiple time points (but AUCs not calculated). The no-effect dose for tumorigenic effects in the mouse carcinogenicity study (60 mg/kg/day) was associated with peak (1 hr) plasma STP levels of ~20 µg/mL. In the rat carcinogenicity study (no evidence of carcinogenic effects), in which the doses were the same as those used in chronic rat toxicity study, the highest dose tested was associated with peak plasma STP levels of ~30-35 µg/mL. Plasma STP levels were not measured in the reproductive and developmental toxicity studies, but drug levels in the rat fertility and peri- and postnatal studies could be reasonably estimated based on the similarity of doses and experimental conditions to the rat general toxicity and carcinogenicity studies. This is not possible for the mouse embryofetal studies (due, e.g., to differences in the age of the studies, labs, animal sources, and vehicles). However, the no-effect dose for increased malformations in mice (50 mg/kg) is below the human dose (50 mg/kg) on a mg/m2 basis.

Toxicology

The toxicology studies of STP predated the ICH guidelines. While the chronic toxicity and carcinogenicity studies were GLP compliant, some of the deficiencies, judged by current standards, include limited TK data and a chronic nonrodent (monkey) toxicity study that was only 6 months in duration. In addition, the Ames test and embryofetal development studies were not conducted according to GLP, and an adequate pre- and postnatal development study with dosing throughout gestation (from implantation) and neurobehavioral testing was not conducted. And, because only limited comparative metabolism data are available, the human relevance of the animal models has not been established.

In the 13-week (CD-1) mouse study, oral (gavage) administration of STP (0, 60, or 800 mg/kg/day) resulted in possibly drug-related deaths and adaptive liver changes (increases in cholesterol, liver weight, and hepatic cell hypertrophy) at the HD (Cmax ~ 60 ug/mL). Peak plasma STP levels at the LD were approximately 15 ug/ml in both sexes.

In the 26-week (S-D) rat study, oral (gavage) administration of STP (0, 80, 220, or 800 mg/kg/day) resulted in deaths, clinical signs, decreased BW, clinical chemistry, urinalysis and macroscopic and microscopic evidence of liver and kidney toxicity at the MD and HD. The liver changes (hepatocellular hypertrophy) appear to have been adaptive and were not considered adverse, but the renal histopathology (increased incidences of tubular nephrosis, tubular basophilia, accumulation of acidophilic globules in the cortical tubular epithelium) associated with increases in plasma urea and electrolytes and proteinuria clearly was adverse. Reversibility was not assessed in this (or any) study. At the no-effect dose, peak plasma levels were approximately 4 and 11 ug/mL in males and females, respectively.

Renal toxicity was also reported in the 4-week (cynomolgus) monkey study, in which oral (gavage) administration of STP (0, 100, 300, or 900 mg/kg/day) resulted in death (1 female), clinical signs (hypotonia, sedation), decreased BW gain, increased blood urea level, increased liver weight, and renal tubular nephrosis at the HD. The no-effect dose (300 mg/kg) was associated with peak plasma STP levels of approximately 24 ug/mL in both sexes.

In the 6-month (cynomolgus) monkey study, oral (gavage) administration of STP (0, 100, 250, or 600 mg/kg/day) resulted in dose-related decreases in RBC parameters at all doses and increased

liver weights and possible renal toxicity (tubulo-interstitial nephropathy) at the MD and HD. While the renal effects were not as pronounced in this study, maximal plasma levels of STP at the HD were only about 20 and 24 ug/mL in males and females (mean values), respectively. The no-effect dose (100 mg/kg) was associated with peak plasma STP levels of approximately 11 ug/mL in both sexes. Effects on RBC parameters (depressed hematopoiesis, neutropenia, thrombocytopenia) have also been noted clinically (see clinical review by Steven Dinsmore).

In in vitro genotoxicity testing, STP was negative in a (non-GLP) Ames test, HPRT gene mutation assay in V79 Chinese hamster cells, UDS assay using mice and rat hepatocytes, and a chromosomal aberration assay in human lymphocytes. In the chromosomal aberration assay in CHO cells, STP concentrations that were considered cytotoxic based on morphological changes (60 and 150 ug/mL) increased incidences of aberrant cells in both the presence and absence of S9. In vivo, STP was negative in the mouse micronucleus test.

Carcinogenicity studies conducted in mice and rats appeared adequate except for possible inappropriate dose selection in the rat study that resulted in excessive BW reductions at the HD. In CD-1 mice, oral (gavage) administration of STP (0, 0, 60, 200, or 600 mg/kg/day) for 78 weeks increased incidences of liver adenomas and carcinomas at the MD and HD. This was not an unexpected result for a demonstrated hepatic enzyme inducer in mice. The different pattern of enzyme induction shown between mice and rats was thought by the sponsor to explain the species difference in tumorigenic response. The no-effect dose for tumorigenic effects in this study was 60 mg/kg/day (1 hr plasma STP levels of ~20 µg/mL).

In S-D rats, oral (gavage) administration of STP (0, 0, 80, 220, or 800 mg/kg/day) for 102 weeks resulted in BW reductions and non-neoplastic adaptive liver changes (hepatocellular hypertrophy and intracytoplasmic concentric inclusions) at the MD and HD but did not produce any clear evidence of carcinogenicity. However, while there was no statistically significant (SS) trend test, pairwise comparisons for some skin tumors in males and females and for testicular tumors in males were SS at the MD compared to C. It is possible that the BW effects at the HD (final BWs 20 and 35% below C in males and females, respectively) altered the dose-response by reducing tumor incidences in that group. The frequencies of certain spontaneous tumors have been shown to correlate positively with mature BW as well as efficiency of food utilization in rats, and BW differences between drug-treated and control groups can mask carcinogenic effects. However, the tumor types increased at the MD in the rat carcinogenicity study of STP are not among those that have been reported to be sensitive to BW changes (Ross, Lustbader, and Bras, 1983; Haseman et al., 1997). In addition, BW was also significantly reduced (final BW 16% below C) in MD females. The renal histopathological finding (tubular nephrosis) prominently observed in the 6-month general toxicity study in rats was not increased in males in this study, presumably due to increased background incidences. However, dose-dependent increases in chronic interstitial tubular nephrosis were seen in females and contracted glomerular tuft was increased in treatment groups of both sexes, as seen in the 6-month study. The highest dose (HD) not associated with tumorigenic effects was associated with peak plasma STP levels of ~30-35 ug/mL.

In a GLP study that was intended to address reproductive performance and pre- and postnatal development, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to male and female S-D rats prior to and during mating and throughout pregnancy and lactation in females did not result in any clear effects on fertility or reproductive performance. The HD induced some maternal/paternal toxicity (clinical signs in females, mortality in 1 male and 2 females, reduced BW gain of males and females). The only developmental toxicity noted was decreased viability of pups during lactation at the HD. No signs of toxicity and no effects on fertility were noted in F1 animals and the development of F2 pups was not affected. This study can be considered an adequate reproductive toxicity assessment, but the numbers of litters examined at term for abnormalities and evaluated postnatally were too small for this to be considered an adequate developmental toxicity (embryofetal or pre- and postnatal) study. In addition, no neurobehavioral evaluations of offspring were conducted.

Three non-GLP mouse embryofetal development (EFD) studies were conducted. In what appeared to be the initial study, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg) to pregnant CD mice throughout organogenesis resulted in increased resorptions and decreased fetal BWs at all doses and an increased incidence of malformations at the HD, with no evidence of maternal toxicity. Cleft palate was seen in 1 MD fetus and 10 fetuses from 2 HD litters. In an effort to follow up on this finding, a study was conducted with only C and HD groups (0 or 800 mg/kg by oral gavage) and an expanded exposure period (GDs 4-14). The results again indicated developmental toxicity (increased mortality, decreased BW, increased malformation incidence) in the absence of maternal toxicity at the single dose tested. Incidences of external malformations (only external examinations were conducted) were increased in the drug-treated group: the number of litters with malformed fetuses (8/22 vs 4/22) and with fetuses with cleft palate (4/22 vs 2/22) was doubled in the drug-treated group compared to C. However, when a third study was conducted in which STP (0, 50, 200, or 800 mg/kg) was given orally (by gavage) to pregnant CD mice throughout organogenesis (GDs 6-16) and only skeletal and visceral fetal examinations were performed, there no effects on incidences of abnormalities.

In a non-GLP oral (gavage) rabbit (wild burgundy strain) EFD study of STP (0, 50, 200, or 800 mg/kg/day), drug-related developmental (increased resorption at MD and HD, decreased fetal BW at all doses) and maternal (decreased BW gain and increased abortion at the MD and HD) toxicity was observed, but there were no apparent drug-related increases in incidences of malformations or variations.

Two studies examining some aspects of pre- and postnatal development were submitted, but neither can be considered adequate. In the first (non-GLP) study, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to female S-D rats from GD 6 through PND 23 resulted in maternal (decreased BW gain at the HD) and developmental toxicity (decreased pup BW at birth and increased mortality during lactation at HD, decreased BW gain during lactation at MD and HD). While the dosing period was adequate, the number of litters per group (10) and the postnatal assessments conducted were not. No neurobehavioral evaluations of offspring were conducted. In the second (GLP) study, oral (gavage) administration of STP (0, 50, 200, or 800 mg/kg/day) to female S-D rats from GD 16 through PND 21 resulted in maternal (mortality, clinical signs, decreased BW gain at the HD) and developmental toxicity (decreased pup survival during PNDs 1-4, decreased pup BW at birth and throughout lactation, deficits in reflex development at HD). While this study used adequate N's (20/grp), the dosing period (starting on GD 16) and the offspring assessments (no reproductive or neurobehavioral evaluations) were inadequate.

A critical deficiency in the nonclinical safety assessment of STP for the intended pediatric indication is the lack of any juvenile animal toxicity studies.

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/s/

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07/27/2018

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07/28/2018