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INITIAL SUBMISSION: FINAL REPORT, DEVELOPMENTAL TOXICITY EVALUATION OF WINGSTAY SN-1 ADMINISTERED BY GAVAGE TO CD (SPRAGUE-DAWLEY) RATS, WITH COVER LETTER DATED 6/16/1998			
Chemical Category			
PROPANOIC ACID, 3-(DODECYLTHIO)-, OXYBIS(2,1-ETHANEDIYLOXY)-*			

INIT  
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MR 7241

# The Goodyear Tire & Rubber Company

8HQ - 0698 - 14206

Akron, Ohio 44316 - 0001

June 16, 1998



BEHQ-98-14206

**Certified Mail**

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Washington, D.C. 20460

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Dear Ladies/Gentlemen:

Subject: TSCA Section 8(e) Notice

Reference: Michael W Smith letter to EPA, May 6, 1998

This submittal does not contain Confidential Business Information.

As promised in the above referenced and attached letter, The Goodyear Tire & Rubber Company is providing the EPA with a copy of the following final report:

Developmental Toxicity Evaluation of Wingstay SN-1. Administered by Gavage to CD (Sprague-Dawley) Rats, Reproductive and Development Toxicology Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, June 4, 1998.

The identity of the test substance is as follows:

Chemical Abstract Name: Propanoic acid, 3-(dodecylthio)-, oxybis(2,1-ethanediyl)oxy-2,1-ethanediyl ester

Chemical Abstract Number: 64253-30-1

My address and telephone number are as follows:



88980000173

98 JUN 24 PM 3:23  
21 FCBIC

The Goodyear Tire & Rubber Company  
Department 100D  
1144 East Market Street  
Akron, Ohio 44316-0001  
Telephone: (330) 796-2362  
Fax: (330) 796-1919

Sincerely,

A handwritten signature in cursive script, appearing to read "Michael W. Smith".

Michael W Smith  
Section Manager, Chemical Information  
Systems & Regulatory Affairs

Attachments (2)

# The Goodyear Tire & Rubber Company

Akron, Ohio 44316-0001

May 6, 1998

## Certified Mail

OPPT Document Processing Center (TS-790)  
Attn: Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics (OPPT)  
U.S. Environmental Protection Agency  
401 M Street, S.W.  
Washington, D.C. 20460

Dear Ladies/Gentlemen:

Subject: **TSCA Section 8(e) Notice**

This submittal does not contain Confidential Business Information.

The Goodyear Tire & Rubber Company is currently sponsoring a study at Research Triangle Institute ( RTI ) in Research Triangle Park, NC to examine the developmental toxicity potential of a rubber additive in laboratory rats. The identity of the test material is as follows:

**CAS Name:** Propanoic acid, 3-( dodecylthio )-, oxybis( 2,1-ethanedioxy-2,1-ethanediyl ) ester

**CAS Number:** 64253-30-1

On April 24, 1998, The Goodyear Tire & Rubber Company received a copy of the draft final report. The conclusions of this draft report ( which may meet EPA's criteria for a substantial risk ) were that exposure to the test material resulted in profound maternal toxicity ( including mortality ) at 540 mg/kg-day, slight maternal toxicity at 180 mg/kg-day, and developmental toxicity, including mortality ( resorptions ), reduced fetal body weights, and teratogenicity at the highest dose of 540 mg/kg-day. Under the requirements of Section 8(e) of the Toxic Substances Control Act and EPA's Statement on Interpretation and Enforcement Policy, 43 Fed.Reg. 1110 ( 16 March 1978 ), The Goodyear Tire & Rubber Company is providing the EPA with a copy of the draft final report.

At the highest dose ( 540 mg/kg-day ), there was 25 % mortality of dams. This dose clearly exceeded the maximum tolerated dose ( MTD ) for both rams and conceptuses. Consequently, the developmental toxicity effects observed at this dose were observed in association with severe maternal toxicity and stress to the dams. The "no observable adverse effect level" ( NOAEL ) for maternal toxicity was 60 mg/kg-day and the NOAEL for developmental toxicity ( including teratogenicity ) was 180 mg/kg-day in rats. Goodyear will provide EPA with a copy of the final report.

Please contact Michael W. Smith, Section Manager, Chemical Information Systems & Regulatory Affairs,  
with any questions:

The Goodyear Tire & Rubber Company  
Department 100D  
1144 East Market Street  
Akron, Ohio 44316-0001

Telephone: (330) 796-2362

Sincerely,

A handwritten signature in black ink, appearing to read "Michael W. Smith". The signature is written in a cursive style with a large, looping initial "M".

Michael W Smith  
Section Manager,  
Chemical Information Systems  
& Regulatory Affairs

*Attachment ( 1 )*



FINAL REPORT

TITLE: Developmental Toxicity Evaluation of Wingstay SN-1 Administered by Gavage to CD® (Sprague-Dawley) Rats

AUTHORS: Rochelle W. Tyl, Ph.D., DABT  
Melissa C. Marr, B.A., LATG  
Christina B. Myers, M.S.

PERFORMING LABORATORY: Reproductive and Developmental Toxicology Laboratory  
Center for Life Sciences and Toxicology  
Chemistry and Life Sciences  
Research Triangle Institute  
P. O. Box 12194  
Research Triangle Park, NC 27709-2194

SPONSOR: The Goodyear Tire and Rubber Company  
Research Division  
142 Goodyear Boulevard  
Akron, Ohio 44305-0001

SPONSOR'S REPRESENTATIVE: Mr. Richard Serva  
Goodyear Tire and Rubber Company

STUDY INITIATION DATE: April 18, 1997

IN-LIFE PERFORMANCE DATES: June 16-July 17, 1997

LABORATORY COMPLETION DATE: September 12, 1997

FINAL REPORT DATE: June 4, 1998

RTI IDENTIFICATION NO.: 65C-6503-400/100

Author:

Rochelle W. Tyl  
Rochelle W. Tyl, Ph.D., DABT  
Study Director  
Life Sciences and Toxicology  
Research Triangle Institute

6/4/98  
Date

Approved:

F. Ivy Carroll  
F. Ivy Carroll, Ph.D.  
Vice President  
Chemistry and Life Sciences  
Research Triangle Institute

6/4/98  
Date

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RTI Project No.: 65C-6503-400  
RTI Protocol No.: RTI-558 (Definitive Study)

Developmental Toxicity Evaluation of Wingstay SN-1  
Administered by Gavage to CD® (Sprague-Dawley) Rats

Sponsor: The Goodyear Tire and Rubber Company

\*\*\*\*\*

ABSTRACT

Based on information provided in the dose range-finding study, timed-pregnant CD® (Sprague-Dawley) rats were exposed to the test chemical, Wingstay SN-1, dissolved in 1% aqueous methyl cellulose and administered by gavage once daily, on gestational days (gd) 6 through 19 at doses of 0, 60, 180 or 540 mg/kg/day (equivalent to 0.0, 6.0, 18.0, and 54.0 mg/ml at a dosing volume of 10.0 ml/kg). There were 25 sperm-positive females per group. The dosing volume was 10.0 ml/kg and was adjusted based on each animal's most recent body weight. Clinical observations were taken daily, except during the dosing period when they were made at least twice daily. Maternal body weights were taken on gd 0, 6, 9, 12, 15, 18, 19 and 20. Feed consumption was measured for the intervals gd 0-6, 6-9, 9-12, 12-15, 15-18, 18-19 and 19-20. At scheduled sacrifice on gd 20, the dams were evaluated for body, liver and gravid uterine weights. Ovarian corpora lutea were counted and the status of uterine implantation sites (*i.e.*, resorptions, dead fetuses, live fetuses) was recorded. All fetuses were dissected from the uterus, counted, weighed, sexed and examined for external abnormalities. Approximately one-half of the live fetuses in each litter were examined for visceral malformations and variations. These fetuses were decapitated and the heads fixed in Bouin's solution; serial free-hand sections of the heads were examined for soft tissue craniofacial malformations and variations. All fetuses in each litter were eviscerated, fixed in alcohol, and stained with alizarin red S/alcian blue. Intact fetuses (approximately one-half per litter, not decapitated) were examined for skeletal malformations and variations.

Pregnancy rates were high and equivalent across all groups (94.4 - 100.0%). Six dams died or were sacrificed moribund at 540 mg/kg/day (two on gd 13, two on gd 14, and one each on gd 16 and 20). Three females (one each at 0, 60 and 540 mg/kg/day) were not pregnant. No dams aborted or delivered early. Three females (two at 60 mg/kg/day and one at 540 mg/kg/day) were removed from study due to intubation (dosing) errors. All pregnant animals at 0, 60 and 180 mg/kg/day had one or more live fetuses at sacrifice; three at 540 mg/kg/day had fully resorbed litters at term. The numbers of litters (and fetuses) examined were 24 (320), 22 (309), 25 (357), and 14 (188) at 0, 60, 180 and 540 mg/kg/day, respectively. Maternal body weights were significantly reduced at 540 mg/kg/day from gd 9 through 20. Maternal weight gain was significantly reduced at 540 mg/kg/day for gd 6-9, for the treatment period, and for maternal gestational weight gain, corrected for gravid uterine weight; gravid uterine weight was also significantly reduced at 540 mg/kg/day. Maternal relative, but not absolute, liver weight was significantly increased at 180 and 540 mg/kg/day. Maternal treatment-related clinical signs were observed at 540 and 180 mg/kg/day. Maternal feed consumption, expressed as g/day, was significantly reduced at 540 mg/kg/day and at 180 mg/kg/day for the dosing and gestational periods and when expressed as g/kg/day at 540 mg/kg/day for the dosing and gestational periods. At 540 mg/kg/day, there were significant increases in the incidence of resorptions (and therefore, nonlive), and of adversely affected (nonlive plus

malformed; due to increases in both resorptions and malformed fetuses) per litter. There were no effects of treatment on preimplantation loss, on the number of live fetuses per litter or on sex ratio (% males per litter). The average fetal body weight per litter (all fetuses or separately by sex) was significantly reduced at 540 mg/kg/day (approximately 80% of the control values). The incidences of litters with one or more fetuses with external, skeletal or any malformations, and the percent fetuses (all fetuses or separately by sex) per litter with malformations were significantly increased at 540 mg/kg/day. External malformations at this dose included failures of closure of neural tube and body wall (dorsal and ventral); failure of caudal development, abnormal eye development and whole body edema. Visceral malformations included hydrocephaly, cardiovascular and pulmonary defects, abdominal hernia, unilateral agenesis of adrenal gland and kidneys, and uni- and bilateral hydronephrosis and hydroureter. Skeletal malformations at this dose included effects on ribs (missing, fused, short [other than XIIIth], and unossified), and thoracic bodies, centra and arches (missing, fused and altered ossification). The incidence of litters with one or more fetuses with external variations was increased at 540 mg/kg/day. There were no treatment-related statistically or biologically significant changes in the incidence of pooled visceral (including craniofacial), skeletal or total fetal variations in this study.

In conclusion, Wingstay SN-1 administered by gavage during major organogenesis in CD® (Sprague-Dawley) rats resulted in profound maternal toxicity (including mortality) at 540 mg/kg/day and slight maternal toxicity at 180 mg/kg/day, and in developmental toxicity including mortality (resorptions), reduced fetal body weights, and teratogenicity at 540 mg/kg/day, a dose which clearly exceeded the maximum tolerated dose for both dams and conceptuses. The "no observable adverse effect level" (NOAEL) for maternal toxicity was 60 mg/kg/day and the NOAEL for developmental toxicity (including teratogenicity) was 180 mg/kg/day in rats under the conditions of this study.

## OBJECTIVES

The present study was designed to evaluate the potential of Wingstay SN-1 to produce maternal and developmental toxicity (including teratogenicity) when administered by gavage during major organogenesis in CD® (Sprague-Dawley) rats.

## MATERIALS AND METHODS

### Test Chemical, Dosage Formulations and Analyses

The test chemical, Wingstay SN-1 (diester of 3-(dodecylthio)propionic acid and tetraethylene glycol; CAS No. 64253-30-1) was received at Research Triangle Institute (RTI) Materials Handling Facility from The Goodyear Tire and Rubber Company, Akron, Ohio, in one shipment with the Sponsor's Lot No. 130893 (Goodyear Notebook No. 10024-68-1). The shipment was received on November 16, 1995 (893.95 and 931.94 g in two 32-ounce plastic bottles) and received the RTI Log Book No. 8394-02-01. The material was a beige, waxy crystalline semi-solid with a garlic odor, and with the chemical purity assumed to be 100.0% for purposes of formulation (see MSDS in protocols, Appendices IV and VI). The bulk material was stored at room temperature under controlled conditions. The vehicle was aqueous (deionized/distilled water; CAS No. 7732-18-5), 0.5% methocel (methyl cellulose, CAS No. 9164-67-5; Fisher Scientific, Springfield, NJ; Lot No. 953456; RTI Log Book No. 8394-26-01) formulated at RTI and stored in amber bottles under refrigeration.

The doses employed were 0, 60, 180 and 540 mg/kg/day, equivalent to 0.0, 6.0, 18.0 and 54.0 mg/ml at a dosing volume of 10.0 ml/kg. Prior to study dose formulation, formulations encompassing the range of doses employed in the study (5.0 and 500.0 mg/ml) were assayed for homogeneity, stability and dose level verification by RTI personnel. Dosing formulations were homogeneous and stable for at least 35 days under refrigeration; dosing suspensions were therefore formulated once during the study and used within the established stability time limit. To prepare dosing formulations, test material was added into 0.5% aqueous methyl cellulose, according to the following formula:

$$\text{Concentration (mg/ml)} = \frac{\text{Dose level (mg/kg)}}{\text{Dose volume (10.0 ml/kg)}}$$

Each dose level was prepared independently.

The Wingstay SN-1 for each dose was stirred and weighed out into a 2 liter glass beaker, and heated slowly to approximately 50-60°C with stirring (at this temperature, Wingstay SN-1 was solubilized). The appropriate volume of 0.5% methyl cellulose was added to each beaker, the stirring bar was removed, the suspension was homogenized for one minute with a Brinkman Polytron homogenizer (setting 3). The resulting formulation was stirred and heated to approximately 50-60°C for removal of analytical and archival samples. After storage and prior to use, the suspensions were heated to approximately 50-60°C, stirred for 30 minutes and homogenized as described above, for one minute. The formulations were stirred and heated during use for dosing.

Dosing formulations with assay values of 90-110% of target were considered to be suitable for use in these studies. Personnel, other than the laboratory supervisor and those involved in the formulation or analyses of dosage formulations, were not informed of the formulation concentrations until all laboratory work had been completed.

A summary of the procedures for preparation of standard solutions, vehicle standards, and dosing formulation samples for analyses follows. Standard solutions were prepared by weighing

Calculations of Standard Developmental Toxicity Parameters

Formulas for calculating standard developmental toxicity parameters are presented in Text Table B.

Text Table B. Formulas for Calculating Standard Developmental Toxicity Parameters<sup>a</sup> (page 1 of 3)

The following endpoints are calculated for each litter (dam) and then the mean is calculated using the litter (dam) values.

1. Percent Preimplantation Loss per Dam and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. corpora lutea} - \text{no. implantation sites}}{\text{no. of corpora lutea}} \right) \text{ per dam} \\ \text{arcsine (square root ((no. corpora lutea} - \text{no. implantation sites)} / \text{no. of corpora lutea}))}$$

2. Percent Resorptions per Litter and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. resorptions in litter}}{\text{no. implantation sites in litter}} \right) \\ \text{arcsine (square root (no. resorptions in litter} / \text{no. implantation sites in litter))}$$

3. Percent Dead per Litter and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. dead in litter}}{\text{no. implantation sites in litter}} \right) \\ \text{arcsine (square root (no. dead in litter} / \text{no. implantation sites in litter))}$$

4. Percent Nonlive per Litter and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. resorptions in litter} + \text{no. dead in litter}}{\text{no. implantation sites in litter}} \right) \\ \text{arcsine (square root ((no. resorptions in litter} + \text{no. dead in litter)} / \text{no. implantation sites in litter))}$$

5. Percent Adversely Affected per Litter and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. resorptions in litter} + \text{no. dead in litter} + \text{no. malformed in litter}}{\text{no. implantation sites in litter}} \right) \\ \text{arcsine (square root ((no. resorptions in litter} + \text{no. dead in litter} + \text{no. malformed in litter)} / \text{no. implantation sites in litter))}$$

6. Percent Males per Litter and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. males in litter}}{\text{no. sexed in litter}} \right) \\ \text{arcsine (square root (no. males in litter} / \text{no. sexed in litter))}$$

7. Average Fetal Body Weight per Litter:

$$\frac{\text{sum of all individual fetal weights in litter}}{\text{no. fetuses weighed in litter}}$$

8. Average Male Fetal Body Weight per Litter:

$$\frac{\text{sum of all individual male fetal weights in litter}}{\text{no. male fetuses weighed in litter}}$$

9. Average Female Fetal Body Weight per Litter:

$$\frac{\text{sum of all individual female fetal weights in litter}}{\text{no. female fetuses weighed in litter}}$$

10. Percent Fetuses with External Malformations per Litter and Arcsine Root Transformation:

$$100 \times \left( \frac{\text{no. fetuses with external malformations in litter}}{\text{no. fetuses examined for external malformations}} \right) \\ \text{arcsine (square root (no. fetuses with external malformations in litter} / \text{no. fetuses examined for external malformations))}$$

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(continued)

**Text Table B. Formulas for Calculating Standard Developmental Toxicity Parameters<sup>a</sup> (page 2 of 3)**

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**11. Percent Fetuses with Visceral Malformations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with visceral malformations in litter} / \text{no. fetuses examined for visceral malformations})$   
 arcsine (square root (no. fetuses with visceral malformations in litter / no. fetuses examined for visceral malformations))

**12. Percent Fetuses with Skeletal Malformations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with skeletal malformations in litter} / \text{no. fetuses examined for skeletal malformations})$   
 arcsine (square root (no. fetuses with skeletal malformations in litter / no. fetuses examined for skeletal malformations))

**13. Percent Fetuses with Malformations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with malformations in litter} / \text{no. fetuses examined for malformations in litter})$   
 arcsine (square root (no. fetuses with malformations in litter / no. fetuses examined for malformations))

**14. Percent Males with Malformations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. males with malformations in litter} / \text{no. males examined for malformations in litter})$   
 arcsine (square root (no. males with malformations in litter / no. males examined for malformations))

**15. Percent Females with Malformations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. females with malformations in litter} / \text{no. females examined for malformations in litter})$   
 arcsine (square root (no. females with malformations in litter / no. females examined for malformations))

**16. Percent Fetuses with External Variations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with external variations in litter} / \text{no. fetuses examined for external variations in litter})$   
 arcsine (square root (no. fetuses with external variations in litter / no. fetuses examined for external variations))

**17. Percent Fetuses with Visceral Variations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with visceral variations in litter} / \text{no. fetuses examined for visceral variations in litter})$   
 arcsine (square root (no. fetuses with visceral variations in litter / no. fetuses examined for visceral variations))

**18. Percent Fetuses with Skeletal Variations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with skeletal variations in litter} / \text{no. fetuses examined for skeletal variations in litter})$   
 arcsine (square root (no. fetuses with skeletal variations in litter / no. fetuses examined for skeletal variations))

**19. Percent Fetuses with Variations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. fetuses with variations in litter} / \text{no. fetuses examined for variations in litter})$   
 arcsine (square root (no. fetuses with variations in litter / no. fetuses examined for variations))

**20. Percent Males with Variations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. males with variations in litter} / \text{no. males examined for variations in litter})$   
 arcsine (square root (no. males with variations in litter / no. males examined for variations))

**21. Percent Females with Variations per Litter and Arcsine Root Transformation:**

$100 \times (\text{no. females with variations in litter} / \text{no. females examined for variations in litter})$   
 arcsine (square root (no. females with variations in litter / no. females examined for variations))

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(continued)

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**Text Table B. Formulas for Calculating Standard Developmental Toxicity Parameters<sup>a</sup> (page 3 of 3)**

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The following endpoints are calculated for each dam and then the mean is calculated using the dam values.

**22. Body Weight Change.**

body weight at end of measurement period - body weight at beginning of measurement period

**23. Feed Consumption in grams per day.**

((feed weight at beginning of measurement period) - (feed weight at end of measurement period)) /  
number of days in measurement period

**24. Feed Consumption in grams per day per kilogram body weight.**

feed consumption in grams per day / average of all body weights taken during measurement period in  
kilograms

**25. Relative Organ Weight.**

(organ weight / sacrifice body weight) x 100

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<sup>a</sup> As required for FDA Good Laboratory Practices (subpart J, paragraph 58.185, no. 11), FIFRA Good Laboratory Practice Standards (subpart J, paragraph 160.185, no. 11) and TSCA Good Laboratory Practice Standards (subpart J, paragraph 792.185, no. 11).

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## Statistics

The unit of comparison was the pregnant female or the litter. Quantitative continuous data (e.g., maternal body weights, fetal body weights, feed consumption, etc.) were compared among the three treatment groups and the vehicle control group by the use of Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances (i.e.,  $p < 0.001$ ), then nonparametric statistical tests were employed for the continuous variables (Winer, 1962; see below). If Bartlett's test indicated homogeneous variances (i.e.,  $p > 0.001$ ), then parametric statistical tests were employed for the continuous variables. Parametric statistical procedures to be applied to selected measures from this developmental toxicity study were as follows. Appropriate General Linear Models (GLM) procedures (SAS Institute Inc., 1989a, 1989b, 1990a, 1990b, 1990c) were used for the Analyses of Variance (ANOVA). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For these litter-derived percentage data, the ANOVA was weighted according to litter size. GLM analysis was used to determine the significance of the dosage-response relationship (Test for Linear Trend), and to determine whether significant dosage effects had occurred for selected measures (ANOVA). When a significant ( $p < 0.05$ ) main effect for dosage occurred, Dunnett's Multiple Comparison Test (Dunnett, 1955; 1964) was used to compare each Wingstay SN-1-exposed group to the control group for that measure. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise differences from the vehicle control group except that a two-tailed test was used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Nonparametric tests to be used on continuous data which did not have homogenous variances included the Kruskal-Wallis Test to determine if significant differences were present among the groups, followed by the Mann-Whitney U test for pairwise differences from the vehicle control group, if the Kruskal-Wallis test was significant (Siegel, 1956). Jonckheere's test for k independent samples (Jonckheere, 1954) was used to identify significant dose-response trends for nonparametric continuous data. Nominal scale measures were analyzed by Chi-Square Test for Independence for differences among treatment groups, and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ( $p < 0.05$ ) differences among groups, then a two-tailed Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise differences between each Wingstay SN-1-dosed group and the control group (Snedecor and Cochran, 1967). A test for statistical outliers (SAS, 1990b) was performed on maternal body weights and feed consumption (in g/day). If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data in g/day were negative for a given dam and period, they were designated "unrealistic" and excluded from summarization and analysis. If feed consumption data for a given observational interval (e.g., gd 6-9, 9-12, 12-15, 15-18 or 18-20 during the treatment period) were designated outliers or unrealistic, then summarized data encompassing this period (e.g., treatment period, gd 6-20) also did not include this value.

## Personnel

The evaluation of Wingstay SN-1 for developmental toxicity in CD® (Sprague-Dawley) rats was conducted at Research Triangle Institute (RTI), Research Triangle Park, NC, under contract to The Goodyear Tire and Rubber Company, Akron, Ohio; Mr. Richard Serva, The Goodyear Tire and Rubber Company, was the Sponsor's Representative. The RTI personnel indicated below contributed to the completion of this study.

Dr. R. W. Tyl served as Study Director. Developmental toxicology personnel included Ms. M. C. Marr (Laboratory Supervisor), Ms. C. B. Myers (Data Specialist), Ms. F. S. Gerling, Ms. V. I. Wilson, Ms. L. B. Pelletier (Study Team), Ms. M-S. Perry, Ms. B. J. McTaggart, Ms. M. V. Cheesborough (Study Team), and Ms. D. A. Wenzel. Bulk chemical handling and dosage

0015

formulations were provided by Mr. M. M. Veselica (Supervisor, MHF), Mr. D. L. Hubbard, Mr. R. A. Price, and Mr. T. D. Burnette. Analyses of dosing formulations were performed by Ms. D. R. Brine, Analytical Chemistry Supervisor, Mr. W. O. Poteat, Mr. J. P. Tan, Ms. J. M. Dewey and Ms. K. E. Amato. Animal care was provided by Dr. D. B. Feldman, DVM, ACLAM, Animal Research Facility (ARF) Veterinarian, and Mr. F. N. Ali, MBA, LATG, ILAM, ARF Manager. Quality Assurance personnel were Ms. S. M. Taulbee, M.S.P.H. (Manager), Ms. C. D. Keller, Ms. P. D. Hall, Mr. S. T. Sherrill, and Ms. M. E. Parker.

The final report was prepared by Dr. R. W. Tyl with assistance from Ms. C. B. Myers and Ms. F. S. Gerling on data compilation and statistical analyses and from Ms. M. C. Marr. The individual scientist reports were prepared and signed by the author(s).

The protocol and two amendments detailing the design and conduct of this study are presented in Appendix IV. The protocol was signed by the Study Director on April 18, 1997.

#### Historical Control Dataset

An historical control summary dataset for developmental toxicity studies with the CD® (Sprague-Dawley) rat in this laboratory is presented in Appendix III.

#### Storage of Records

All original data sheets for the present study are stored in the RTI archives, under the control of the RTI Chemistry and Life Sciences Archivist, along with all biological samples collected during the course of the study which remain the responsibility of RTI. Work sheets and computer printouts which were generated in the statistical analysis of data are stored in the RTI Archives. Copies of this report are filed in the RTI Chemistry and Life Sciences Archives as well as with The Goodyear Tire and Rubber Company, Akron, OH.

#### Compliance

The study was performed in compliance with the Toxic Substances Control Act (TSCA) testing guidelines (U.S. EPA, 1985), the OPPTS draft guidelines (U.S. EPA, 1996), and, to the extent possible, in compliance with the Japanese Ministry of International Trade and Industry (MITI) Handbook of Existing and New Chemical Substances, Sixth Edition, V. Teratogenicity Test (1993), and the OECD teratogenicity testing guidelines (OECD, 1981a).

All records, biological samples, data and reports will be maintained in storage as specified in the TSCA GLPs (U.S. EPA, 1989) or for as long as the quality of the preparation affords evaluation, whichever is less.

The toxicology laboratories at RTI are operated in compliance with TSCA Good Laboratory Practice Standards (GLPs) (U.S. EPA, 1989) and the OECD Principles of GLPs (OECD, 1981b). The RTI Animal Research Facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. This study was conducted in compliance with the TSCA GLP regulations and AAALAC accreditation standards.

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## RESULTS

### Dosing Formulations

The dosing formulations were homogeneous and stable under refrigeration (protected from light) for at least 35 days. Dosing suspensions were therefore formulated once and used throughout the dosing period. They were considered acceptable for use if the analytical values were  $\pm 10\%$  of target concentrations. Concentrations of Wingstay SN-1 in the dosing suspensions were 96.0-108% of target upon pre-dosing analysis and no test chemical was detected in the vehicle control suspension with the estimated limit of detection of 0.24 mg/ml (Table 1 and Appendix I).

### Maternal Toxicity

Pregnancy rates were high and approximately equivalent across all groups (94.4 - 100.0%) (Table 2). Three females were removed from study due to technician dosing error (misdirected dose), two at 60 mg/kg/day and one at 540 mg/kg/day. Six females died or were sacrificed moribund at 540 mg/kg/day, two on gd 13, two on gd 14, one on gd 16 and one on gd 20. Three females (one each at 0, 60 and 540 mg/kg/day) were nonpregnant in the study. No dams aborted or delivered early. All pregnant dams at 0, 60, and 180 mg/kg/day had one or more live fetuses at scheduled sacrifice; three at 540 mg/kg/day carried fully resorbed litters at term. The numbers of litters (and fetuses) evaluated were 24 (320) at 0 mg/kg/day, 22 (309) at 60 mg/kg/day, 25 (357) at 180 mg/kg/day, and 14 (188) at 540 mg/kg/day. Maternal body weights were equivalent across all groups for gd 0 and 6 prior to the onset of dosing. Starting on gd 9, mean body weights at 540 mg/kg/day were statistically significantly reduced for all timepoints, gd 9, 12, 15, 18 and 19. Maternal body weights on gd 20 (in-life and at sacrifice) were also significantly reduced at 540 mg/kg/day. Maternal weight change was equivalent across all groups for gd 0-6, prior to the onset of dosing. Maternal weight change was significantly reduced at 540 mg/kg/day for gd 6-9, the first interval of the dosing period, and was equivalent across all groups for all subsequent intervals encompassing gd 9 through 20. Maternal weight change during treatment (gd 6-20) and maternal gestational weight change (gd 0-20) were significantly reduced at 540 mg/kg/day. Maternal gestational weight change corrected for the weight of the gravid uterus was also significantly reduced at 540 mg/kg/day. Gravid uterine weight was significantly reduced at 540 mg/kg/day (due, at least in part, to the three fully resorbed litters at this dose). Maternal absolute liver weight was equivalent across all groups; maternal liver weight, relative to terminal body weight, was significantly increased at 180 and 540 mg/kg/day (Table 2). Individual maternal body and organ weights are presented in Appendix II.

Clinical observations are presented in Table 3. Treatment-related clinical observations were present almost exclusively at 540 mg/kg/day. Rust colored fur on neck, nose, and/or feet, a non-specific indicator of stress (from hemoglobin breakdown products in the Harderian gland excreted via the tear ducts, so-called "bloody tears" or chromodacryorrhea, groomed from the face), was first observed on gd 6 (post-dosing) at 540 mg/kg/day through gd 19; at 180 mg/kg/day, it was observed first on gd 12 through 14; at 60 mg/kg/day only on gd 14; chromodacryorrhea per se (on face) was observed at 540 mg/kg/day on gd 10, 12-15 and 17, and at 180 mg/kg/day only on gd 19. Piloerection, another non-specific sign of stress, was observed at 540 mg/kg/day on gd 7, 9-20, at 180 mg/kg/day on gd 12, 13, 15-20 and at 60 mg/kg/day on gd 16, 18 and 19, in dose-related patterns of incidence and time to presence. Rough coat (more severe than piloerection, indicative of more stress) was observed only at 540 mg/kg/day on gd 8-15 and 20. Urine-stained fur around urogenital area was observed at 540 mg/kg/day on gd 11-13. Rooting in bedding post-dosing (most likely due to taste aversion and not toxicity per se) was observed at 60 mg/kg/day on gd 9, at 540 mg/kg/day on gd 12-19, and at 180 mg/kg/day on gd 14-19. Lethargy was observed at 540 mg/kg/day on gd 12-20. Hunched posture was observed only at 540 mg/kg/day on gd 12-14, and 16-20. Dehydration was also observed at 540 mg/kg/day on gd 10-15 and 17-20. At 540 mg/kg/day, one dam was found dead and one dam was euthanized moribund on gd 13, two dams were

## DISCUSSION

The present study has shown that Wingstay SN-1, administered by gavage during major organogenesis (gd 6 through 19) in CD® (Sprague-Dawley) rats resulted in profound maternal toxicity at 540 mg/kg/day. Effects at this dose included mortality (six of 23 pregnant, 26.1%), reduced maternal body weights and weight changes, including maternal gestational weight change corrected for gravid uterine weight (which is an indicator of maternal toxicity *per se*, unconfounded by any effects on embryofetal number or size), clinical signs of toxicity, and reduced maternal feed consumption throughout the dosing and gestational periods. Maternal toxicity at 180 mg/kg/day was limited to reduced feed consumption during the dosing and gestational periods, and occasional incidences of clinical observations consistent with stress. Increased relative (but not absolute) liver weight was observed at 180 and 540 mg/kg/day; however this finding may be due to induction of hepatic metabolizing enzymes and concomitant increase in liver mass, and not to toxicity *per se* (Conney, 1967). There was no evidence for maternal toxicity at 60 mg/kg/day. The high dose, 540 mg/kg/day, clearly exceeded the maximum tolerated dose for the dams. See Text Table C (Part A) for a summary of indicators of maternal toxicity.

Developmental toxicity was observed only at 540 mg/kg/day and included increased resorptions (three out of 17 litters at this dose were fully resorbed), and therefore increased nonlive (resorbed plus dead), and increased incidence of adversely affected implants (nonlive plus malformed) per litter due to increased incidences of resorptions and malformations. The percentages of litters with one or more fetuses with external, skeletal, or any malformations were significantly increased at 540 mg/kg/day. (The percentage of litters with one or more fetuses with visceral malformations was increased at 540 mg/kg/day, 28.57%, versus the control value, 8.33%, but the difference was not statistically significantly different.) The percentages of fetuses with malformations per litter, for all fetuses or separately by sex, were also significantly increased at 540 mg/kg/day. The values for the parameter, percentage of litters with one or more affected fetuses, indicated that more litters were affected at 540 mg/kg/day: two, three, two and six litters with one or more malformed fetuses at 0, 60, 180 and 540 mg/kg/day, respectively. The values for the parameter, percentages of fetuses per litter with malformations, indicated that there were more fetuses per litter affected at 540 mg/kg/day: 0.13, 0.18, 0.8 and 1.57 fetuses with one or more malformations per litter at 0, 60, 180 and 540 mg/kg/day, respectively. The external malformations could be grouped as failures of closure of the neural tube and of the dorsal body wall (exencephaly, meningoencephalocele, meningocele, soft skin protuberance on back), abnormalities of caudal development (anal atresia and short threadlike tail), failure of ventral body wall closure (gastroschisis), abnormalities of eye development (uni- and bilateral anophthalmia and unilateral microphthalmia), and whole body edema (anasarca) in fetuses with other external malformations. The visceral malformations, observed only at 540 mg/kg/day, included hydrocephaly, cardiovascular defects (common truncus and dextrocardia in two different fetuses in the same litter), pulmonary malformations (enlarged right lung lobe, missing postcaval lung lobe, in the fetus with common truncus), abdominal hernia (also observed in the range-finding study, Appendix V), unilateral (right) agenesis of adrenal gland and kidney in one fetus, and another fetus in the same litter with unilateral agenesis of the left kidney. Uni- and bilateral hydronephrosis and/or uni- and bilateral hydroureter, were observed at a low frequency in all groups. These are the most common visceral malformations observed in the performing laboratory's historical control animals (Appendix III) and in published historical control databases (e.g., Charles River, 1988; Woo and Hoar, 1979).

Skeletal malformations, observed almost exclusively at 540 mg/kg/day, involved the ribs (missing, fused, short rib other than rib XIII, unossified), vertebrae involving the bodies, centra and arches of the vertebrae (missing, fused and bipartite cartilage of the centra). Most of the affected

fetuses in the affected litters at 540 mg/kg/day exhibited multiple malformations (except for one fetus in one litter, fetus no. 6 of dam no. 65, with only one malformation), originating from various embryonic cell layers, and initiated early (e.g., CNS and eye lesion) or later during major organogenesis (e.g., anal atresia, skeletal effects, hydroureter, hydronephrosis) with no apparent single specific time or target. This dose clearly exceeded the maximum tolerated dose for conceptuses, in the presence of profound maternal toxicity.

External variations were observed only at 540 mg/kg/day (and therefore exhibited a significant increase at this dose relative to the control value) and included only clubbed limb without bone change (not present in the historical control dataset, Appendix III); visceral variations, observed in all groups, were predominantly enlarged lateral ventricles of the cerebrum, associated with small fetuses (most likely delayed development due to toxicity; very common in historical controls), displaced gonads (both ovary and testes), agenesis of the innominate artery (found in historical control fetuses) and distended ureters (also common in historical control fetuses). Skeletal variations, observed in all groups, included extra rib in Lumbar I, short XIIIth rib, misaligned sternbrae and reduced ossification in vertebral centra (all common in historical control fetuses, Appendix III). See Text Table C (Part B) for a summary of indicators of developmental toxicity.

In conclusion, Wingstay SN-1 administered by gavage during major organogenesis in CD® (Sprague-Dawley) rats resulted in profound maternal toxicity (including mortality) at 540 mg/kg/day and slight maternal toxicity at 180 mg/kg/day, and in developmental toxicity including mortality (resorptions), reduced fetal body weights, and teratogenicity at 540 mg/kg/day, a dose which clearly exceeded the maximum tolerated dose for both dams and conceptuses. The "no observable adverse effect level" (NOAEL) for maternal toxicity was 60 mg/kg/day and the NOAEL for developmental toxicity (including teratogenicity) was 180 mg/kg/day in rats under the conditions of this study.

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Text Table C  
Summary of Maternal and Developmental Toxicity

	Wingstay SN-1, mg/kg/day, po			
	0	60	180	540
<b>A. Maternal</b>				
Mortality	---	---	---	↑(6) <sup>a</sup>
Body weights	---	---	---	↓
Weight change	---	---	---	↓
Clinical observations	---	---	↑ <sup>a</sup>	↑↑ <sup>a</sup>
Feed consumption	---	---	↓	↓
Gravid uterine weight	---	---	---	↓
Liver weight, absolute	---	---	---	---
Liver weight, relative	---	---	↑	↑
<b>B. Developmental</b>				
Preimplantation loss	---	---	---	---
Postimplantation loss	---	↓	---	↑ (n.s.) <sup>b</sup>
Resorptions	---	↓	---	↑ (n.s.) <sup>b</sup>
Adversely affected implants per litter <sup>c</sup>	---	---	---	↑
No. live fetuses/litter	---	---	---	---
Fetal sex ratio (% males)	---	---	---	---
Fetal body weight	---	---	---	↓
Fetal malformations				
External	---	---	---	↑
Visceral	---	---	---	↑(n.s.)
Skeletal	---	---	---	↑
All	---	---	---	↑
Fetal variations				
External	---	---	---	↑
Visceral	---	---	---	---
Skeletal	---	---	---	---
All	---	---	---	---

## KEY:

↑ = statistically significant increase

↓ = statistically significant decrease

n.s. = not statistically significant

<sup>a</sup> = the incidences of maternal mortality and of clinical observations were not analyzed for statistical significance; a double arrow for clinical observations at 540 mg/kg/day indicates more profound clinical observations (and/or in more dams) at this dose than at 180 mg/kg/day.

<sup>b</sup> = three litters (of 24 pregnant) were fully resorbed at this dose.

<sup>c</sup> = adversely affected implants includes resorbed implants and dead fetuses (nonlive) plus malformed fetuses.

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