E02187.01 Technical Report Tables

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TABLE 1: Experimental Design and Materials and Methods for the Embryo/Fetal Development Feed Study of Oxybenzone

Experimental Design and Materials & Methods	
Study Information	
Study Laboratory	National Center for Toxicological Research (NCTR), 3900 NCTR Road, Jefferson, AR 72079
Test Article	Oxybenzone (OXY)
Synonyms:	2-hydroxy-4-methoxybenzophenone, HMB, benzophenone-3, (2-hydroxy-4-methoxyphenyl)-phenylmethanone
CAS No.	131-57-7
Purity	>99%
Supplier	Ivy Fine Chemicals, Cherry Hill, NJ [catalog number: HH13-026; lot #: 1F100604]
Dates of Study Initiation and Completion	Protocol Approved: April 6, 2012 (In-Life) Initiation: July 9, 2012 Completion: September 14, 2012 Final Report Signed: September 21, 2015
Animals and Animal Maintenance	
Species/Strain/Substrain	Rat/Sprague-Dawley/Harlan
Animal Source	Harlan Industries (Indianapolis, IN)
Receiving Date	Load 1: July 9, 2012 Load 2: July 16, 2012 Load 3: August 13, 2012 Load 4: August 20, 2012 Load 5: August 27, 2012
Date of First Exposure	Load 1: July 12, 2012 Load 2: July 19, 2012 Load 3: August 16, 2012 Load 4: August 23, 2012 Load 5: August 30, 2012
Date of Last Exposure	Load 1: July 21, 2012 Load 2: July 28, 2012 Load 3: August 25, 2012 Load 4: September 1, 2012 Load 5: September 8, 2012

Experimental Design and Materials & Methods	
Age and Weight of Animals upon Receipt	Time-mated females were approximately 11-13 weeks old upon receipt. Animals were 200-240 grams at the time of mating.
Acclimation Time before Start of Test	3 days – gestational day (GD) 3 animals were allocated to the study on arrival and placed on a soy- and alfalfa-free diet (Purina 5K96).
Method of Allocation	Time-mated females were allocated to exposure groups by a stratified randomization procedure to give groups approximately the same initial mean body weight.
Animal Identification	Tail tattoo with cage number.
Method of Euthanasia for Dams	Carbon dioxide asphyxiation.
Method of Euthanasia for Fetuses	Decapitation or by an intraperitoneal injection of Euthasol followed by bilateral thoracotomy.
Feed	Irradiated Purina 5K96 chow (Test Diets, Purina Mills, Inc., St. Louis, MO), available *ad libitum*. Each lot was analyzed by the Chemistry Support Group, Division of Biochemical Toxicology, NCTR, Jefferson, AR.
Water	Millipore®-filtered tap water (Jefferson, AR municipal supply) via water bottles, available *ad libitum*.
Animals per Cage	All animals were housed individually.
Cages	Solid-bottom polysulfone cages (Allentown Caging Equipment Co., Allentown, NJ), changed weekly.
Bedding	Irradiated heat-treated hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly.
Cage Bonnets	Microisolator tops (Lab Products, Inc., Maywood, NJ), changed every three weeks.
Racks	Metal animal cage racks (Allentown Caging Equipment Co., Allentown, NJ), changed every three weeks.
Animal Room Environment	Temperature: 23°C ± 3°C Relative humidity: 50% ± 20% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour

Experimental Design and Materials & Methods	
Sentinel Animals/Microbiological Surveillance	Sentinel animals (a total of four) were maintained in the animal room utilized in the study and were evaluated during the course of the study and at study completion. Animal room supplies (food, water, bedding) and swabs from the animal rooms were also evaluated. An additional ten animals (two per shipment/five shipments) were ordered for microbiology surveillance. These animals were tested upon arrival. Sentinel and surveillance animals were shared with NTP protocol E02188.01. E02187.01 and E02188.01 ran concurrently; study animals for both protocols arrived in the same shipments.
Experimental Design	
Size of Study Groups	Twenty-five time-mated females per treatment group.
Doses/Route of Exposure	0; 3,000; 10,000; 30,000 ppm oxybenzone in the feed (5K96), available *ad libitum.*
Duration of Exposure	9 days – morning of GD 6 until the morning of GD 15
Type and Frequency of Observations	Dams - Observed twice daily for morbidity and mortality; clinical signs recorded once daily. Body weights recorded on GD 3, 6, 10, 14, 17 and 21; food consumption recorded at least twice weekly and at the start and stop of dosing.
Caesarean Section	GD 21
Fetal Examinations	All live fetuses evaluated for internal, external and skeletal effects; heads evaluated from approximately 50% of fetuses by Wilson's free-hand razor dissection.
Additional Evaluations Conducted	At necropsy the abdominal and thoracic cavities of the dams were examined for gross lesions. Organ weights for the liver, kidneys (separate) and ovaries (separate) as well as the gravid uterine weights were obtained from the dams. The number and status of each implantation site was recorded. For any female that appeared non-pregnant, the uterus was stained with 10% ammonium sulfide. The number of viable and dead fetuses, sex and fetal weights were also recorded for each litter. Corpora lutea counts were performed for each ovary. Blood samples for hematological and clinical chemistry analysis were collected from 10 randomly selected dams in each treatment group. Hematological measurements included counts for white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and red blood cells; percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils; determination of hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, the hematocrit, platelet counts and packed cell volume. Clinical chemistry measurements included determination of sorbitol dehydrogenase, total bile acids, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, triglyceride, cholesterol, total protein, creatine kinase, creatinine, blood urea nitrogen and glucose levels.

Experimental Design and Materials & Methods	
Test Article Vehicle Mixture	
Mixture Preparation	Weighed amounts of oxybenzone were mixed with Purina 5K96 chow on an as needed basis. Doses were prepared by the Diet Preparation Group, Priority One Services, NCTR, Jefferson, AR.
Stability	Oxybenzone was found to be stable in Purina 5K96 chow through seven weeks[1] at refrigerated temperature in E0217801 [Dose-Finding Oxybenzone – NTP; non-GLP].
Storage Conditions of Test Article	Stored in the original containers(s) at room temperature.
Storage Conditions of Dose Formulations	Dose formulations were stored in stainless steel cans secured with tie-downs at 4°C ± 2°C.

[1]Stability at the time the protocol was approved was six weeks. Refer to the Analytical Chemistry Report (Appendix IV) for data supporting stability of seven weeks.

TABLE 2: Maternal Body Weights (g) of Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Gestational Day (GD)	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
6	237.0 ± 2.8 (19)	236.5 ± 2.6 (21)	236.7 ± 2.6 (22)	239.9 ± 2.8 (19)
10* **	254.0 ± 1.4 (19)	251.2 ± 1.3 (21)	247.6 ± 1.3** (22)	239.6 ± 1.4*** (19)
14* **	274.2 ± 1.8 (18)	270.1 ± 1.7 (21)	263.7 ± 1.6*** (21)	254.3 ± 1.8*** (18)
17 **	306.8 ± 2.6 (19)	299.7 ± 2.6 (21)	295.2 ± 2.4** (22)	294.5 ± 2.6** (19)
21*	352.7 ± 3.3 (19)	349.9 ± 3.2 (21)	342.5 ± 3.1 (22)	342.0 ± 3.3 (19)
6 - 21* **	284.9 ± 1.6	281.5 ± 1.5	277.1 ± 1.5**	274.0 ± 1.6***

ANOCOVA results: Treatment, p = < 0.001; GD, p = < 0.001; Treatment x GD, p = < 0.001; Baseline weight, p = < 0.001.

[a] Body weight analysis was adjusted for baseline weight at GD 3 prior to dosing at GD 6.

[b] Mean body weight (g) ± S.E.M. Numbers in parentheses indicate number of pregnant dams per treatment group. Asterisks (*) adjacent to gestational day in shaded cells indicate significant trends in least square mean comparisons of maternal body weight. Asterisks adjacent to means in shaded cells indicate significant pairwise differences at the same gestational day(s) during pregnancy as determined by Dunnett's method for adjusted contrasts. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

TABLE 3: Summary Statistics of Maternal Interval Body Weight Gain (g) of Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Gestational Day (GD)	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
3-6	21.11 ± 1.51 (19)	21.06 ± 1.73 (21)	21.24 ± 2.03 (22)	24.61 ± 4.73 (19)
6-10	16.97 ± 1.17 (19)	14.75 ± 2.13 (21)	10.90 ± 1.91 (22)	-0.26 ± 4.49 (19)
10-14	19.67 ± 1.03 (18)	18.86 ± 1.14 (21)	16.11 ± 1.06 (21)	14.83 ± 1.06 (18)
14-17	30.98 ± 1.14 (18)	29.66 ± 2.37 (21)	31.48 ± 1.45 (21)	40.80 ± 1.65 (18)
17-21	46.56 ± 2.87 (19)	50.18 ± 4.60 (21)	47.26 ± 1.50 (22)	47.18 ± 2.51 (19)

[a] Animals were fed control chow from GD 3 until the morning of GD 6; dosed chow from the morning to GD 6 to the morning of GD 15; control chow from the morning of GD 15 until sacrifice at GD 21.
[b] Summary interval mean body weight gain (g) ± S.E.M. Numbers in parentheses indicate number of pregnant dams per treatment group. Statistical analysis was not performed on the data set.

TABLE 4: Daily Food Consumption (g/animal/day) of Rats Administered Dietary Oxybenzone from GD 6 to GD 15[a,b]

Feed Consumption (g/animal/day)	Dietary Oxybenzone (ppm)			
	CTRL	**OXY 3,000**	**OXY 10,000**	**OXY 30,000**
GD 6-10* **	17.7 ± 1.8 (19)	18.5 ± 1.7 (21)	15.7 ± 1.7 (22)	29.6 ± 1.8*** (19)
GD 10-14* **	20.3 ± 1.8 (19)	19.2 ± 1.7 (21)	18.4 ± 1.7 (22)	34.6 ± 1.8*** (19)
GD 14-17* **	21.4 ± 1.2 (19)	22.6 ± 1.1 (21)	20.7 ± 1.1 (22)	26.7 ± 1.2** (19)
GD 17-21* **	25.5 ± 1.1 (19)	24.0 ± 1.0 (21)	24.3 ± 1.0 (22)	29.6 ± 1.1* (19)
GD 6-21* **	21.2 ± 0.9 (19)	21.1 ± 0.9 (21)	19.8 ± 0.8 (22)	30.1 ± 0.9*** (19)

[a] Dams were fed control chow from arrival at GD 3 until the morning of GD 6; dosed chow from the morning of GD 6 to the morning of GD 15; control chow from the morning GD 15 until sacrifice at GD 21.
[b] Mean daily food consumption (g/animal/day) ± S.E.M. reported by treatment group. Numbers in parentheses indicate number of pregnant dams per treatment group.
[c] ANOVA results: Treatment, $p = <0.001$; GD, $p = <0.001$; Treatment x GD, $p = <0.001$.
Asterisks (*) adjacent to food consumption designation in shaded cells indicate significant trends in least square mean comparisons of maternal food consumption. Asterisks adjacent to means in shaded cells indicate significant pairwise differences at the same gestational days from controls during pregnancy as determined by Dunnett's method for adjusted contrasts. *, $p < 0.05$; **, $p < 0.01$; ***, $p \leq 0.001$.

TABLE 5: Estimated Ingested Doses of Oxybenzone in Rats Administered 0, 3,000, 10,000 or 30,000 ppm in Feed from GD 6 to GD 15[a]

Dosing Period	Mean Dose (mg/kg body weight per day) ± S.E.M.			
	CTRL	OXY 3,000[b]	OXY 10,000[b]	OXY 30,000[b]
GD 6-15	0.0 ± 0.0	242.3 ± 7.9 [205.8 – 399.7]	724.6 ± 28.3 [560.8 – 905.8]	3688.5 ± 426.0 [1816.9 – 5990.9]

[a] The estimated mean ingested dose for the dosing period (GD 6 -15) was calculated by multiplying the dietary concentration of oxybenzone by the mean measured amount of food ingested at GD 6, GD 10 and GD 14 and dividing the result by the mean body weight for the corresponding gestational day. The means for GD 6, GD 10 and GD 14 were then averaged to determine an approximate mean ingested dose (mg/kg body weight per day).
[b] Numbers in brackets indicate the range of estimated ingested doses. Ranges shown are averages of GD 6, GD 10 and GD 14 data determined from individual animals.

TABLE 6: Maternal Organ Weights (g) of Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a]

Organ Weight (g)	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
Liver[c]*	10.41± 0.22 (19)	10.23 ± .20 (21)	10.82 ± 0.20 (22)	10.95 ± 0.21 (19)
Kidney Paired[b,d]	1.50 ± 0.03 (19)	1.44 ± 0.02 (21)	1.48 ± 0.02 (22)	1.47 ± 0.03 (19)
Ovary Paired[b,e]	0.17 ± 0.01 (19)	0.18 ± 0.01 (21)	0.17 ± 0.01 (22)	0.18 ± 0.01 (19)

[a] Mean organ weight (g) ± S.E.M. reported by treatment group. Numbers in parentheses indicate number of pregnant dams per treatment group.
[b] Analysis was conducted on the combined weight of paired organs.
[c] ANOCOVA results: Wt. at sacrifice, p = <0.001; Treatment, p = 0.058.
[d] ANOCOVA results: Wt. at sacrifice, p = <0.001; Treatment, p = 0.349.
[e] ANOCOVA results: Wt. at sacrifice, p = 0.049; Treatment, p = 0.753.
Asterisks (*) adjacent to organ weight designation in shaded cells indicate significant trends in least square mean comparisons of maternal organ weights. *, $p < 0.05$. Pairwise comparisons were performed with Dunnett's method for adjusted contrasts; no significant pairwise differences were observed between control and treatment groups.

TABLE 7: Summary Statistics of Maternal Organ Weights (g) of Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Organ Weight (g)	Dietary Oxybenzone (ppm)			
	CTRL (19)	**OXY 3,000** (21)	**OXY 10,000** (22)	**OXY 30,000** (19)
Liver	10.70 ± 0.25	10.34 ± 0.25	10.65 ± 0.23	10.75 ± 0.21
Kidney, Left	0.76 ± 0.02	0.73 ± 0.01	0.74 ± 0.01	0.72 ± 0.01
Kidney, Right	0.76 ± 0.02	0.72 ± 0.01	0.73 ± 0.01	0.73 ± 0.01
Kidney, Paired	1.52 ± 0.04	1.45 ± 0.02	1.47 ± 0.03	1.45 ± 0.02
Ovary, Left	0.087 ± 0.007	0.086 ± 0.004	0.080 ± 0.005	0.085 ± 0.005
Ovary, Right	0.087 ± 0.004	0.096 ± 0.004	0.091 ± 0.004	0.088 ± 0.004
Ovary Paired	0.174 ± 0.008	0.181 ± 0.005	0.172 ± 0.007	0.173 ± 0.006
Body Weight at Sacrifice[c]	352.7 ± 4.5	347.8 ± 3.7	339.6 ± 3.1	338.7 ± 3.2

[a] Summary mean (g) ± S.E.M. reported by treatment group. Numbers in parentheses indicate number of pregnant dams per treatment group.
[b] Paired values represent actual means of the summed values for each animal.
[c] Mean body weight (g) of dams at time of sacrifice.

TABLE 8: Summary Statistics of Maternal Organ Weights (mg) to Body Weight (g) Ratios of Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a]

Organ Weight (g)	Dietary Oxybenzone (ppm)			
	CTRL (19)	**OXY 3,000** (21)	**OXY 10,000** (22)	**OXY 30,000** (19)
Liver	30.34 ± 0.62	29.67 ± 0.48	31.39 ± 0.70	31.72 ± 0.51
Kidney, Left	2.16 ± 0.05	2.09 ± 0.03	2.17 ± 0.04	2.12 ± 0.03
Kidney, Right	2.16 ± 0.05	2.08 ± 0.03	2.16 ± 0.04	2.16 ± 0.03
Kidney, Paired	4.32 ± 0.09	4.17 ± 0.06	4.32 ± 0.07	4.29 ± 0.06
Ovary, Left	0.244 ± 0.019	0.246 ± 0.010	0.237 ± 0.015	0.252 ± 0.015
Ovary, Right	0.249 ± 0.013	0.276 ± 0.011	0.268 ± 0.011	0.259 ± 0.012
Ovary Paired	0.493 ± 0.021	0.522 ± 0.013	0.505 ± 0.019	0.511 ± 0.018

[a] Summary mean (g) ± S.E.M. reported by treatment group. Numbers in parentheses indicate number of pregnant dams per treatment group.

TABLE 9: Hematological Parameters of Dams Exposed to Dietary Oxybenzone from GD 6 to GD 15[a]

Hematological Endpoint [ANOVA p value][b]	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
WBC (10^3/mm^3) [p = 0.540]	4.09 ± 0.56	2.92 ± 0.31	3.58 ± 0.47	3.46 ± 0.57
NEU (%) [p = 0.143]	32.48 ± 2.34	39.70 ± 2.02	36.89 ± 2.62	36.09 ± 1.86
LYM (%) [p = 0.380]	53.25 ± 2.94	46.51 ± 2.35	48.19 ± 2.74	47.45 ± 1.95
MON (%) [p = 0.297]	13.45 ± 0.97	13.18 ± 1.03	13.98 ± 0.96	15.65 ± 1.19
EOS (%) [p = 0.260]	0.67 ± 0.09	0.47 ± 0.09	0.81 ± 0.16	0.66 ± 0.09
BAS (%) [p = 0.970]	0.15 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.15 ± 0.02
NEU (10^3/mm^3) [p = 0.918]	1.30 ± 0.16	1.13 ± 0.11	1.31 ± 0.21	1.22 ± 0.19
LYM (10^3/mm^3) [p = 0.278]	2.22 ± 0.36	1.38 ± 0.19	1.78 ± 0.30	1.67 ± 0.31
MON (10^3/mm^3) [p = 0.395]	0.54 ± 0.08	0.38 ± 0.05	0.47 ± 0.04	0.53 ± 0.09
EOS (10^3/mm^3) [p = 0.470]	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
BAS (10^3/mm^3) [p = 0.500]	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RBC (10^6/mm^3)* [*p = 0.049*]	6.30 ± 0.09	6.01 ± 0.11	6.01 ± 0.10	5.97 ± 0.06*
HGB (g/dL) [p = 0.059]	12.34 ± 0.15	11.72 ± 0.27*	11.84 ± 0.18	11.73 ± 0.15*
HCT (%) [*p = 0.034*]	34.98 ± 0.41	33.08 ± 0.77*	33.37 ± 0.52*	33.16 ± 0.50*
MCV (µm^3) [p = 0.786]	55.60 ± 0.45	55.10 ± 0.53	55.60 ± 0.31	55.70 ± 0.45
MCH (pg) [p = 0.772]	19.57 ± 0.15	19.51 ± 0.17	19.70 ± 0.13	19.68 ± 0.10
MCHC (g/dL) [p = 0.446]	35.23 ± 0.08	35.44 ± 0.14	35.47 ± 0.09	35.42 ± 0.14
PLT (10^3/mm^3)** [*p = 0.022*]	1044.0 ± 29.6	1113.8 ± 24.6	1087.6 ± 44.2	1233.2 ± 51.3**
PCV (%) [p = 0.062]	35.00 ± 0.45	33.30 ± 0.86	33.60 ± 0.53	33.15 ± 0.51*

Abbreviations: WBC = white blood cells; NEU = neutrophils; LYM = lymphocytes; MON = monocytes; EOS = eosinophils; BAS = basophils; RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; PCV = packed cell volume

[a] Summary mean ± S.E.M. reported per treatment group. Ten pregnant dams from each treatment group were randomly selected for hematological/clinical chemistry analysis.
[b]ANOVA results are presented in brackets for each hematological parameter analyzed. Analysis that indicated significant treatment effects are shown in italics; significance level was set at $p = 0.05$.
Asterisks (*) adjacent to hematological endpoints in shaded cells indicate significant trends in least square mean comparisons of oxybenzone treatments to the control. Asterisks adjacent to means in shaded cells indicate significant pairwise differences from controls as determined by Dunnett's method for adjusted contrasts. *, $p < 0.05$; **, $p < 0.01$.

TABLE 10: Clinical Chemistry Parameters of Dams Exposed to Dietary Oxybenzone from GD 6 to GD 15[a]

Clinical Chemistry Endpoint [ANOVA p value][b]	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
SDH (U/L) [p = 0.937]	7.73 ± 1.88	7.61 ± 2.15	7.39 ± 1.10	11.04 ± 3.35
TBA (µmol/L)** [*p = 0.017*]	42.24 ± 7.86	69.29 ± 13.78	62.28 ± 21.45	90.07 ± 9.64**
ALB (g/dL) [p = 0.480]	2.97 ± 0.07	2.87 ± 0.06	2.97 ± 0.07	2.85 ± 0.10
ALT (U/L) [p = 0.202]	56.80 ± 3.33	52.90 ± 3.50	49.70 ± 3.00	58.30 ± 2.91
ALP (U/L) [p = 0.492]	111.50 ± 11.40	90.30 ± 8.31	94.20 ± 10.17	96.40 ± 8.32
AST (U/L) [p = 0.960]	78.30 ± 4.91	80.10 ± 7.05	78.40 ± 5.44	88.70 ± 10.20
TRIG (mg/dL) [p = 0.683]	194.50 ± 44.41	198.60 ± 51.14	172.50 ± 30.42	277.10 ± 68.12
CHOL (mg/dL)** [*p = 0.007*]	119.90 ± 4.19	116.40 ± 3.73	123.20 ± 3.71	138.30 ± 4.13*
TP (g/dL) [p = 0.322]	5.63 ± 0.11	5.38 ± 0.09	5.49 ± 0.13	5.28 ± 0.14
CK (U/L) [p = 0.170]	263.50 ± 34.74	203.80 ± 33.10	199.70 ± 39.57	255.40 ± 50.02
CREAT (mg/dL) [p = 0.441]	0.58 ± 0.04	0.53 ± 0.04	0.55 ± 0.02	0.51 ± 0.04
BUN (mg/dL)* [p = 0.065]	17.50 ± 0.78	16.10 ± 0.71	18.70 ± 1.27	15.60 ± 0.60*
GLU (mg/dL) [p = 0.085]	92.80 ± 2.71	84.20 ± 5.24	100.90 ± 5.89	84.30 ± 5.60

Abbreviations: SDH = sorbitol dehydrogenase; TBA = total bile acids; ALB = albumin; ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; TRIG = triglycerides; CHOL = cholesterol; TP = total protein; CK = creatine kinase; CREAT = creatinine; BUN = blood urea nitrogen; GLU = glucose

[a] Summary mean ± S.E.M. reported per treatment group. Ten pregnant dams from each treatment group were randomly selected for hematological/clinical chemistry analysis.
[b] ANOVA results are presented in brackets for each clinical chemistry parameter analyzed. Analysis that indicated significant treatment effects are shown in italics; significance level was set at p = 0.05.
Asterisks (*) adjacent to clinical chemistry endpoints in shaded cells indicate significant trends in least square mean comparisons of oxybenzone treatments to the control. Asterisks adjacent to means in shaded cells indicate significant pairwise differences from controls as determined by Dunnett's method for adjusted contrasts. *, $p < 0.05$; **, p = 0. 001.

TABLE 11: Reproductive/Pregnancy Parameters of Dams Exposed to Dietary Oxybenzone from GD 6 to GD 15

Endpoint	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
No. Pregnant[a,f]	19 (76.0%)	21 (84.0%)	22 (88.0%)	19 (76.0%)
No. Not Pregnant	6	4	3	6
Gravid Uterine Weights (g)[b,g,j]	86.9 ± 3.0	90.0 ± 2.6	84.9 ± 4.2	92.6 ± 3.1
Corpora Lutea[b]	14.8 ± 0.4	15.0 ± 0.3	14.6 ± 0.5	15.1 ± 0.5
Implantation Sites[b,h,j]	13.1 ± 0.4	13.4 ± 0.5	12.6 ± 0.6	13.7 ± 0.5
Resorptions[b,c,i,j]	0.6 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.2
Live[b]	12.5 ± 0.5	13.0 ± 0.5	12.3 ± 0.6	13.4 ± 0.6
% Pre-Implantation Loss[d]	11.4 ± 2.4	10.9 ± 2.8	12.5 ± 3.8	8.4 ± 2.1
% Post-Implantation Loss[e]	4.6 ± 2.0	2.8 ± 1.4	2.8 ± 1.1	3.1 ± 2.1

[a] Numbers in parentheses indicate percentage of pregnant females per treatment group.
[b] Summary mean ± S.E.M. reported by treatment group.
[c] All resorptions were classified as early.
[d] Pre-implantation loss was defined as the percentage of corpora lutea that did not result in implantation.
[e] Post-implantation loss was defined as the percentage of implantations that were resorbed.
[f] Fisher's Exact test was used for comparison of treatments to control; the Cochran-Armitage test was used for analysis of trend across treatments. No significant differences by either test were identified.
[g] Results of a one-way ANOVA: Treatment, $p = 0.380$.
[h] Counts of implantation sites were analyzed using Poisson regression with terms for treatment and covariate number of corpora lutea. There was no significant treatment effect; the covariate corpora lutea was significant ($p = 0.011$).
[i] Counts of resorptions were analyzed using Poisson regression with terms for treatment and covariate number of implantation sites. There was no significant treatment or covariate effect.
[j] Pairwise comparisons were performed with Dunnett's method for adjusted contrasts. Tests were conducted as two-sided at the 0.05 significance level; no significant pairwise differences were observed between control and treatment groups.

TABLE 12: Litter Parameters of Dams Exposed to Dietary Oxybenzone from GD 6 to GD 15[a]

Endpoint	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000[b]	OXY 10,000	OXY 30,000[b]
Total Live Fetuses[c,i]	12.53 ± 0.81 (19)	13.00 ± 0.79 (21)	12.27 ± 0.75 (22)	13.37 ± 0.84 (19)
Male Live Fetuses[d,i]	6.37 ± 0.58 (19)	6.76 ± 0.57 (21)	6.05 ± 0.52 (22)	6.79 ± 0.60 (19)
Female Live Fetuses[d,i]	6.16 ± 0.57 (19)	6.24 ± 0.55 (21)	6.23 ± 0.53 (22)	6.58 ± 0.59 (19)
Total Litter Weight[e,i]	65.36 ± 0.80 (19)	65.59 ± 0.76 (20)	64.85 ± 0.74 (22)	65.97 ± 0.80 (18)
Male Fetal Weight[f,i]	5.25 ± 0.07 (19)	5.27 ± 0.07 (21)	5.26 ± 0.07 (22)	5.28 ± 0.07 (19)
Female Fetal Weight[g,i]	5.02 ± 0.08 (19)	5.03 ± 0.08 (21)	4.93 ± 0.08 (22)	5.09 ± 0.08 (19)
Proportion Males[h,i]	0.51 ± 0.03 (19)	0.52 ± 0.03 (21)	0.49 ± 0.02 (22)	0.51 ± 0.04 (19)

[a] Mean ± S.E.M. reported per litter. Numbers in parentheses indicate number of litters per treatment group.
[b] One fetus in each of the OXY 3,000 and OXY 30,000 ppm treatment groups was not sexed.
[c] Counts of total live fetuses were analyzed using Poisson regression. Total live analysis was based on the sum of unsexed and sexed pups.
[d] Counts of total male and female live fetuses were analyzed using Poisson regression. Unsexed pups were classified as male for analysis.
[e] ANOCOVA results: Treatment, $p = 0.777$, Litter size, $p = <0.001$. For analysis of litter weight, weight was combined across sex including unsexed fetuses.
[f] Results of a one-way ANOVA: Treatment, $p = 0.992$. Analysis of weight by sex was performed using data of weighed male fetuses. The two unsexed fetuses were not included in the analysis.
[g] Results of a one-way ANOVA: Treatment, $p = 0.593$. Analysis of weight by sex was performed using data of weighed female fetuses. The two unsexed fetuses were not included in the analysis.
[h] Proportion of male fetuses within litters was analyzed for treatment effects using logistic regression. Unsexed fetuses were assigned as male for analysis.
[i] Pairwise comparisons were performed with Dunnett's method for adjusted contrasts. Tests were conducted as two-sided at the 0.05 significance level; no significant pairwise differences were observed between control and treatment groups.

TABLE 13: Visceral Variations and Malformations in Fetal Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Visceral Variations/Malformations	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
Total[c]*	0.634 ± 0.183 (19)	0.821 ± 0.195 (21)	0.920 ± 0.206 (22)	1.351 ± 0.267 (19)
Dilated Ureter**	0.526 ± 0.166 (19)	0.632 ± 0.171 (21)	0.733 ± 0.184 (22)	1.230 ± 0.256 (19)

[a] Mean ± S.E.M. reported per litter. Numbers in parentheses indicate number of litters per treatment group.
[b] Analyses of fetal visceral variations/malformations were adjusted for litter size.
[c] Total counts of visceral variations/malformations is the sum of cleft palate, dilated ureter, malpositioned testes and other variations/malformations. The category of other was defined as variations/malformations that were observed in only one fetus and included increased intestines, lung discoloration, enlarged liver or major anomalies.
Asterisks (*) adjacent to visceral variations/malformations designation in shaded cells indicate significant trends. Single symbol, $p < 0.05$; double symbol, $p = 0.01$. Pairwise comparisons were performed with Dunnett's method for adjusted contrasts; no significant pairwise differences were observed between control and treatment groups.

TABLE 14: Total Number of Visceral Variations/Malformations in Fetal Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Visceral Evaluations	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
Number of Fetuses Examined	238	273	270	254
Total Number of Visceral Findings[c]	12	18	20	28
Number of Litters	19	21	22	19
Number of Litters with Visceral Findings	7	9	8	9
Dilated Ureter (Total)[d]	**10 (6)**	**14 (9)**	**16 (7)**	**26 (9)**
Left	6 (5)	9 (7)	7 (4)	16 (8)
Right	0	1 (1)	0	3 (3)
Bilateral	4 (3)	4 (3)	9 (5)	7 (5)
Percent Dilated Ureter Variations[d,e]	**4.5%**	**5.3%**	**5.4%**	**9.8%**
Cleft Palate	**0**	**0**	**1 (1)**	**2 (1)**
Malpositioned Testis	**1 (1)**	**2 (2)**	**1 (1)**	**0**
Other (Total)	**1 (1)**	**2 (2)**	**2 (2)**	0
Increased intestines	1(1)	0	0	0
Lung discoloration	0	1 (1)	1(1)	0
Enlarged liver	0	0	1 (1)	0
Major anomalies	0	1 (1)	0	0

[a] Each variation/malformation was summed across litters within a treatment group.
[b] Numbers in parentheses indicate number of litters affected per treatment group.
[c] Total is the sum of all dilated ureters, cleft palate, malpositioned testis and other variations/malformations within a treatment group.
[d] Observed dilated ureters were mild or moderate and grouped together for analysis.
[e] For each litter, percentage was calculated as the number of fetuses with variations/malformations divided by the number of live fetuses.

TABLE 15: Skeletal Variations in Fetal Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Skeletal Variation	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
Total[c]	1.576 ± 0.298 (18)	2.811 ± 0.366* (21)	2.162 ± 0.315 (22)	1.480 ± 0.278 (19)
Ribs*	1.176 ± 0.257 (18)	1.795 ± 0.293 (21)	1.146 ± 0.230 (22)	0.622 ± 0.181 (19)
Sternebrae	0.057 ± 0.057 (18)	0.139 ± 0.080 (21)	0.598 ± 0.166 (22)	0.299 ± 0.125 (19)
Centra	0.339 ± 0.138 (18)	0.838 ± 0.200 (21)	0.368 ± 0.130 (22)	0.555 ± .0.171 (19)
Number of Fetuses with Skeletal Variations	1.464 ± 0.287 (18)	2.485 ± 0.344 (21)	2.024 ± 0.305 (22)	1.379 ± 0.268 (19)

[a] Mean ± S.E.M. reported per litter. Numbers in parentheses indicate number of litters per treatment group.
[b] Analyses of fetal variations were adjusted for litter size.
[c] Total counts of skeletal variations is the sum of rib, sternebrae, centra and other variations. The category of other was defined as variations/malformations that were observed in only one fetus and included a shortened tail and multiple major anomalies.
Asterisks adjacent to skeletal variations in shaded cells indicate significant trends: *, $p < 0.05$. Asterisks adjacent to means in shaded cells indicate significant pairwise differences from controls as determined by Dunnett's method for adjusted contrasts: *, $p < 0.05$.

TABLE 16: Total Number of Skeletal Variations in Fetal Rats Exposed to Dietary Oxybenzone from GD 6 to GD 15[a,b]

Skeletal Evaluations	Dietary Oxybenzone (ppm)			
	CTRL	OXY 3,000	OXY 10,000	OXY 30,000
Number of Fetuses Examined	222	273	270	254
Total Number of Skeletal Findings[c]	28	60	47	29
Number of Fetuses with Skeletal Findings	26	53	44	27
Total Number of Litters	18	21	22	19
Number of Litters with Skeletal Findings	10	17	18	9
Percent Total Variations[c,d]	**11.8%**	**19.3%**	**16.8%**	**10.6%**
Rib (Total)[e]	**21 (10)**	**38 (13)**	**25 (12)**	**12 (7)**
Left - Rudimentary	11 (7)	11 (7)	11 (7)	5 (4)
Right -Rudimentary	4 (3)	11 (5)	8 (7)	2 (2)
Bilateral -Rudimentary	5 (4)	14 (8)	5 (4)	5 (3)
Thoracic Rib – 14th	1 (1)	2 (1)	1 (1)	0
Percent Rib Variations[d]	**9.3%**	**14.0%**	**9.5%**	**4.7%**
Sternebrae (Total)[e]	**1 (1)**	**3 (2)**	**13 (7)**	**6 (4)[#]**
No Ossification	0	1 (1)	0	0
Decreased Ossification	0	1 (1)	9 (3)	4 (2)
Misaligned	0	0	4 (4)	3 (3)
Split	1 (1)	1 (1)	0	0
Percent Sternebrae Variations[d]	**0.4%**	**1.0%**	**5.2%**	**2.4%**
Centra (Total)[e]	**6 (4)**	**18 (9)**	**8 (5)**	**11 (5)[##]**
Split	2 (2)	3 (2)	1 (1)	5 (3)
Dumbbell-shaped	4 (3)	15 (8)	7 (5)	7 (4)
Percent Centra Variations[d]	**2.8%**	**6.5%**	**3.0%**	**4.2%**
Other (Total)[e]	**0**	**1 (1)**	**1 (1)**	0
Multiple major anomalies, one fetus	0	1 (1)	0	0
Shortened Tail	0	0	1 (1)	0

[a] Each variation was summed across litters within a treatment group.
[b] Numbers in parentheses indicate number of litters affected per treatment group.
[c] Total is the sum of all rib, sternebrae, centra and other variations within a treatment group.
[d] For each litter, percentage was calculated as the number of fetuses with variations divided by the number of live fetuses.
[e] Numbers indicate the number of fetuses with variations; numbers in parentheses indicate number of litters affected.
[#] One fetus had a single sternebra that had decreased ossification and was misaligned, only counted as one variation in the total count.
[##] One fetus had two centra that were dumbbell-shape.