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2-METHYLHEXANOIC ACID DEVELOPMENTAL TOXICITY TESTING

by

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Abstract: As part of an investigation of the developmental effects and structure-activity relationships of aliphatic acids, 2-methylhexanoic acid was administered by gavage to Sprague-Dawley rats on gestation days 6-15 at doses of 0, 300, and 400 mg/kg/day. The dams were allowed to deliver and their litters were examined through postnatal day 6. Pups that were found dead were examined for soft-tissue alterations. On day 6, two survivors per litter were preserved for skeletal examination. Maternal toxicity was demonstrated at both 300 and 400 mg/kg by weight loss and altered respiration (rales, dyspnea). In spite of the maternal toxicity present, there were no clear toxic effects on development; litter size, pre- and postnatal viability, and pup weights were unaffected by treatment. Skeletal examinations of selected pups yielded inconclusive data; a slight increase in the incidence of lumbar ribs was present at 400 mg/kg, but was not clearly attributable to treatment.

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INTRODUCTION

Valproic acid (VPA), an anticonvulsant, is teratogenic in humans and rodents (Lammer et al., '87). We are using the rat to investigate the developmental effects and structure-activity relationships of VPA and related chemicals. Studies with VPA, butanoic, 2-ethylbutanoic, pentanoic, 2-methylpentanoic, 2-ethylhexanoic, 3-methylhexanoic, 5-methylhexanoic, octanoic, and 2-methyloctanoic acids were reported previously (Narotsky et al., '88, '89a, '89b; Mervin et al., '89a, '89b, '89c; Narotsky and Kavlock, '89, '90a, '90b, '91). In this effort, we investigated 2-methylhexanoic acid (2MH) using an *in vivo* developmental toxicity screen developed by Chernoff and Kavlock ('82).

MATERIALS AND METHODS

Chemical

The test compound was 2-methylhexanoic acid (99%, Aldrich Chemical Co., Milwaukee, WI, Lot No. 03612EV). Corn oil (laboratory grade, Fisher Scientific, Co.) was used as the vehicle. Solutions were prepared at appropriate concentrations to provide the desired dose when administered at 1 ml solution/kg body weight.

Animal Husbandry

Timed pregnant 90-day-old Sprague-Dawley rats, 240-280 grams, were obtained from Charles River Laboratories, Raleigh, NC and individually housed in 18x9½x8" polycarbonate cages. Heat-treated wood shavings were supplied for bedding. The animals were provided feed (Purina Laboratory Rodent Chow #5001) and tap water *ad libitum* and a 12-hr. photoperiod (lights on at 0600). Room temperature and relative humidity were maintained at 72±2°F and 50±10%, respectively.

Procedures

Dose-Range-Finding Study:

In order to determine appropriate dose levels for the present study, a preliminary study was conducted using nongravid females of the same age and strain. Six females, individually housed, were assigned to each of five groups receiving 0, 300, 600, 900, or 1200 mg 2MH/kg/day for 10 days; dosing solutions were administered by gavage at 2 ml/kg initial body weight. Animals were maintained for 5 days posttreatment. Body weights were determined on study days 1, 3, 5, 8, 11, and 15 and clinical observations were recorded.

Developmental Toxicity Screen:

The day that evidence of mating (e.g., sperm in the vaginal smear) was detected was designated gestation day (GD) 0. On GD 3, the rats were assigned to one of three treatment groups using a nonbiased procedure that assured a homogeneous distribution of body weights among groups. Twenty, 15, and 15 rats were gavaged daily on GD 6-15 at dosages of 0, 300, and 400 mg 2MH/kg, respectively, at a volume of 1 ml solution/kg body weight. All doses were based on individual GD-6 body weights. Maternal body weights were determined on GD 3, 6, 8, 10, 13, 16, and 20 and the rats were examined throughout the experimental period for clinical signs of toxicity.

Beginning on GD 20, the dams were observed periodically (up to seven times per day) to determine the stage (completed, in progress, or first pup delivered) and time of parturition. Postnatal day (PD) 1 was defined as GD 22 independent of the actual time of parturition. Pups in each litter were counted on PD 1, 3, and 6 and were weighed collectively on PD 1 and 6. Pups that were found dead with no gross malformations were preserved in Bodian's solution (2% formaldehyde, 5% acetic acid, 72% ethanol) and examined for soft-tissue alterations. On PD 6, two survivors (one/sex, if possible) from each litter were fixed in 65% ethanol and stained with alizarin red S for skeletal examination. Soft-tissue examinations included examination of the head using free-hand sections similar to that described by Wilson ('65). The thoracic and abdominal viscera were examined by dissection. After PD 6, the dams were killed and uterine implantation sites were counted. Females that did not deliver were killed on presumed GD 24 and their uteri were examined to determine pregnancy status.

Statistics

Data were analyzed using the General Linear Models procedure on SAS. Since the *a priori* hypothesis was that treatment could only reduce the number of surviving progeny, one-tailed tests were used for pertinent data. Gestation-length data were analyzed using the Kruskal-Wallis test; ranks were based on the time and stage that parturition was observed. The number of live PD-1 pups was used as a covariate in the analysis of pup weights. Similarly, the number of implants was used as a covariate in the analyses of the number of live pups. When a significant treatment effect was detected by analysis of variance, Student's t-test on least-squares means was used to identify individual groups that were significantly different from the control group.

Prenatal loss for each litter was defined as the number of implants that were not viable at the PD-1 examination. Postnatal loss was defined as the number of implants that were viable on PD 1, but not on PD 6. An additional parameter, perinatal loss, was defined as the number of implants that did not survive to PD 6.

Dams with only one implant were excluded from statistical analyses. Fully resorbed litters were regarded to have zero live pups at all postnatal examinations. Litters that were delivered after PD 1 were included in the PD-3, PD-6, and perinatal loss analyses, but not in the PD-1, pre-, and postnatal loss analyses. Pup-examination data were considered anecdotal and were not statistically analyzed.

RESULTS

Dose-Range-Finding Study

Body weight losses were evident after two doses at 1200 mg/kg and after seven doses at 300 and 600 mg/kg (Table 1). Evaluation of body weight patterns at 900 mg/kg was confounded by high mortality in this group. Signs of respiratory toxicity (usually rales) were present in all animals treated with 2MH.

Dyspnea was evident in one female in each of the groups receiving 600, 900, and 1200 mg/kg. Motor depression (ataxia or decreased motor activity) was observed in two females at 600 mg/kg and three females at 1200 mg/kg. Death occurred in one 300-mg/kg female (day 8), in three females at 900 mg/kg (days 2, 3, and 5), and in one female at 1200 mg/kg (day 4). All animals survived at 600 mg/kg.

Based on the results of this dose-range-finding study, dose levels of 0, 300, and 400 mg/kg were selected for the developmental toxicity screen.

Maternal Data

All females survived the experimental period; however, maternal toxicity was demonstrated by signs of respiratory toxicity (dyspnea, rales, vocalization, nasal congestion) in most females of both dose groups (Table 2, Appendix 1). Ataxia was observed in two (13%) high-dose females within four hours after dosing on the ninth day of treatment.

Body weight losses after the first 2 days of dosing were significant at both dose levels (Table 2, Appendix 2). Mean body weights decreased 0.5 and 10.3 g. for the low- and high-dose groups, respectively; whereas control dams gained 9.8 g. over the first 2 days of treatment. Significant dose-related reductions in weight gain were evident in both dose groups after 4 doses and on GD 20. Gestational weight gains adjusted for live-litter weights were also affected in a dose-related manner and were significantly reduced in the high-dose group.

Developmental Data

Gestation lengths, litter sizes, pre- and postnatal viability, and pup weights were comparable in all groups (Table 3, Appendix 3). Perinatal mortality was marginally increased in the low-dose group due to the death of one litter (#2033) with four pups. High-dose values for perinatal mortality were comparable to controls.

Visceral examinations were conducted on two pups from low-dose litter #2033. Both pups, killed moribund on PD 3, had a dilated ureter; one pup also had a dilated renal pelvis. The remaining two pups in this litter died after PD 3 and were apparently cannibalized. An additional pup in each of the control and high-dose groups was found dead, but autolysis precluded a complete examination of these specimens. Skeletal examinations conducted on two PD-6 survivors from each litter revealed slightly increased incidences of lumbar ribs in the high-dose group (Table 3). Virtually all the lumbar ribs were rudimentary (i.e., focal). The only exception was a short rib (less than one-half the length of the adjacent rib) observed in a high-dose pup.

DISCUSSION

Maternal toxicity was demonstrated at both 300 and 400 mg/kg by weight loss early in the treatment period and by respiratory effects. Isolated cases of ataxia and reduced gestational weight gains adjusted for PD-1 litter weights

Despite these maternal effects, no clear developmental toxicity was evident at either dose level. There were no delays in parturition, nor any definitive effects on progeny growth or viability. A marginal increase in perinatal mortality at 300 mg/kg was attributed to the postnatal death of one litter (#2033) with four pups. The cause of this litter's death was undetermined; but, due to the nondose-related pattern, we regard this finding to be unrelated to treatment.

A slight increase in the incidence of lumbar ribs in selected PD-6 pups at 400 mg/kg suggested a developmental effect similar to that of VPA and 2-ethylhexanoic acid (2EH). VPA, in a Segment II study (Narotsky et al., '88), and 2EH, in an *in vivo* screen (Narotsky et al., '89a), both induced lumbar ribs. However, unlike the present study, full-length lumbar ribs were observed. In addition, VPA and 2EH were also associated with cervical ribs, fused ribs, fused vertebrae, extra presacral vertebrae, and missing caudal vertebrae. Since only a small number of randomly selected pups from each litter were examined in the present study, and no other evidence of developmental toxicity was present at this dose level, these skeletal data were considered inconclusive.

In a comparison of structure-activity relationships of 2MH and the ten other aliphatic acids studied in our laboratory thus far (Narotsky et al., '88, '89a, '89b; Mervin et al., '89a, '89b, '89c; Narotsky and Kavlock, '89, '90a, '90b, '91), all induced maternal respiratory toxicity. However, only 3-methylhexanoic acid, 2EH, and VPA (2-propylpentanoic acid) caused pronounced motor depression (e.g., ataxia, lethargy) in the developmental toxicity screen. 2MH and 2-methyloctanoic acid also caused motor depression, but with much lower incidence. Only VPA and 2EH were associated with dramatic effects on development; these two compounds caused delayed parturition in addition to malformations of the vertebrae and ribs (see above). Structurally, 2MH differs from the developmentally toxic 2EH, only by possessing a one-carbon side chain. Thus, the lack of developmental toxicity for 2MH demonstrates the strict structural restraints for the biological activities for these congeners.

A qualitative summary of results obtained in this project to date is presented in Table 4. Additional testing with other aliphatic acids as well as halogen- and alkyloxy-substituted compounds will be conducted to gain further insights in the structure-activity relationships of this class of chemicals and their effects on development.

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Table 1: Summary of Dose-Range-Finding Study of Nongravid Rats Gavaged for 10 Days With 2-Methylhexanoic Acid.

	DOSE LEVEL (mg/kg/day)				
	0	300	600	900	1200
No. Rats					
Treated	6	6	6	6	6
Affected					
Rales	0	5	5	6	5
Dyspnea	0	0	1	1	1
Ataxia	0	0	1	0	2
Dec. Activity	0	0	1	0	1
Death	0	1	0	3	1
Mean ± S.E. (n) Body Weights (g)					
Day 1	273.7 ± 3.9(6)	273.0 ± 4.8(6)	270.8 ± 3.1(6)	275.8 ± 6.7(5)[a]	273.7 ± 6.7(6)
Day 3	275.7 ± 3.8(6)	276.5 ± 4.5(6)	272.8 ± 5.2(6)	276.6 ±12.1(5)	267.3 ±10.2(6)
Day 5	279.3 ± 4.8(6)	279.5 ± 4.5(6)	275.5 ± 3.9(6)	276.0 ± 7.6(4)	267.6 ±13.6(6)
Day 8	282.8 ± 6.4(6)	277.8 ± 3.8(6)	270.8 ± 5.0(6)	279.0 ±10.7(3)	268.4 ±13.4(5)
Day 11	284.7 ± 7.1(6)	287.4 ± 5.7(5)	275.8 ± 5.1(6)	282.0 ±18.2(3)	263.2 ±10.6(5)
Day 15	294.2 ± 7.8(6)	292.8 ± 5.0(5)	289.7 ± 4.4(6)	306.3 ±19.4(3)	278.8 ± 8.9(5)

a Animal that died prior to Day 3 was excluded from the calculations.

Table 2: Maternal Data for Rats Gavaged With 2-Methylhexanoic Acid on Gestation Days (GD) 6-15.

	DOSE LEVEL (mg/kg/day) 0	300	400
No. Females			
Treated	20	15	15
Affected (%)			
Dyspnea	0	1 (7)	1 (7)
Rales	1	13 (87)	15 (100)
Vocalization	0	5 (33)	9 (60)
Ataxia	0	0	2 (13)
Died	0	0	0
With 1 Implant	0	0	0
Remaining Pregnant	17	11	13
Maternal Body Weights (g)			
Day 6	278.2 ± 2.0[i]	280.1 ± 3.8	283.7 ± 2.4
Day 8	288.1 ± 2.3	279.6 ± 6.7	273.4 ± 5.7[a]
Day 10	300.3 ± 2.2	287.8 ± 7.5[a]	289.9 ± 3.8[a]
Day 13	321.8 ± 2.7	308.7 ± 6.8[a]	308.9 ± 4.6[a]
Day 16	351.6 ± 3.1	333.1 ± 7.1*	327.7 ± 7.6**
Day 20	414.3 ± 4.6	394.1 ± 5.9*	389.8 ± 8.2*
Maternal Weight Gains (g)			
Days 6- 8	9.8 ± 1.3	-0.5 ± 4.1*	-10.3 ± 5.0***
Days 6-10	22.1 ± 1.7	7.7 ± 5.6**	6.2 ± 3.2**
Days 10-16	51.3 ± 2.2	45.3 ± 3.6	37.8 ± 7.2
Days 16-20	62.7 ± 2.1	61.0 ± 3.4	62.2 ± 2.3
Days 6-16	73.4 ± 2.5	53.0 ± 4.5**	44.0 ± 7.6***
Days 6-20	136.1 ± 4.0	114.0 ± 4.3*	106.2 ± 8.3**
Days 6-20 (adj[e])	52.7 ± 3.6	41.2 ± 4.9	31.6 ± 6.8*

a Marginally significant difference from control value ($p \leq 0.10$).
* Significantly different from control value ($p \leq 0.05$).
** Significantly different from control value ($p \leq 0.01$).
*** Significantly different from control value ($p \leq 0.001$).

e Adjusted for the live-litter weight on postnatal day 1. The sample size is the number of dams with live pups on postnatal day 1 (see Table 3).
i Mean ± S.E. using the number of females "remaining pregnant" as the sample size.

Table 3: Developmental Data for Rats Gavaged With 2-Methylhexanoic Acid on Gestation Days 6-15.

	DOSE LEVEL (mg/kg/day) 0	300	400
No. Dams	17	11	13
Delivering			
Gestation Day 21	16	10	11
Gestation Day 22	1	1	2
With Live Pups: Day 1	17	11	13
Day 6	17	10	12[b]
No. Implants[j]	13.0 ± 0.4[i]	11.5 ± 0.9	12.6 ± 0.6
No. Live Pups: Day 1[k]	11.8 ± 0.4	9.9 ± 1.0	10.8 ± 0.6
Day 3[j]	11.8 ± 0.4	9.9 ± 1.0	10.7 ± 0.6
Day 6[j]	11.7 ± 0.4	9.5 ± 1.3	10.7 ± 0.7[b]
Percent Loss: Prenatal[k]	9.3 ± 2.1	14.2 ± 4.5	14.2 ± 3.4
Postnatal[k]	0.5 ± 0.5	9.1 ± 9.1	0.6 ± 0.6[b]
Perinatal[j]	9.8 ± 2.0	23.2 ± 8.8[a]	14.6 ± 3.6[b]
Perinatal Mortality (No.)[j]	1.3 ± 0.3	2.0 ± 0.5	1.9 ± 0.5[b]
Pup Weight (g): Day 1[m]	7.1 ± 0.1	7.5 ± 0.3	7.0 ± 0.2
Day 6	14.1 ± 0.3	15.4 ± 0.8	14.0 ± 0.6[b]
Skeletal Findings[o]:			
No. Pups (Litters)			
Examined	34 (17)	20 (10)	26 (13)
Affected			
Rudimentary Lumbar Rib	8 (6)	4 (4)	11 (7)
Extra Sternebra	4 (4)	1	0
Percent Pups (Litters)			
Affected			
Rudimentary Lumbar Rib	24 (35)	20 (40)	42 (54)
Extra Sternebra	12 (24)	5 (10)	0
Mean ± S.E. Percent/Litter			
Rudimentary Lumbar Rib	23.5 ± 8.7	20.0 ± 8.2	42.3 ±12.5
Extra Sternebra	11.8 ± 5.3	5.0 ± 5.0	0

a Marginal significant difference from control value ($p \leq 0.10$).
b Day-6 data from Dam 2020 excluded from calculations due to malfunctioning water spigot.
i Mean ± S.E.
j Sample size: the total number of dams (i.e., 17, 10, and 13 for the control, low-, and high-dose groups, respectively).
k Sample size: the number of litters examined on Day 1 (i.e., 17, 11, and 13 for the control, low-, and high-dose groups, respectively).
m Sample size: the number of dams with live pups on the day specified.
o Two PD-6 pups were examined from each litter.

Table 4: Summary of Results of Developmental Toxicity Assays With Aliphatic Acids.

Aliphatic Acid	Maternal Effects: Motor Depression	Maternal Effects: Altered Respiration	Maternal Effects: Weight Loss	Developmental Effects: Delayed Parturition	Developmental Effects: Reduced Pup Weight	Developmental Effects: Decreased Pup Viability	Developmental Effects: Malformations
Butanoic	-	+	+	-	+[a]	+[a]	-
2-Ethyl-butanoic	-	+	+	-	-	-	-
Pentanoic[e]	-	+	+	-	±[b]	-	-
2-Methyl-pentanoic	-	+	+	-	-	-	-
2-Propyl-pentanoic[e]	+	+	+	+	+	+	+
2-Ethyl-hexanoic	+	+	+	+	+	+	+
2-Methyl-hexanoic	+	+	+	-	-	-	-
3-Methyl-hexanoic	+	+	+	-	-	-	-
5-Methyl-hexanoic	-	+	+	-	-	-	-
Octanoic	+[d]	+	+	-	-	+[a]	-
2-Methyl-octanoic	+	+	+	-	-	-	-

a Occurred only in dams with peripartum respiratory symptoms.
b Positive in Segment II study, negative in screen.
d In dose-range-finding study.
e Results of segment II study included.

APPENDIX 1

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL MATERNAL OBSERVATIONS

CONTROL

RAT	Ataxia	Dyspnea	Rales	Vocal-ization	Nasal Congestion	Fluid Around Nose	Sali-vation
2012							
2013							
2014							
2016							
2019							
2024							
2026							
2028			K		e		
2032							
2036							
2037							
2040							
2042							
2043							
2044							
2047							
2049							
2051							
2055							
2056							

Note: Each character indicates the day the condition was observed:
A = GD6, B = GD7, C = GD8... 1 = PD1, 3 = PD3, 6 = PD6.
A lowercase letter indicates the condition was observed only within the first 4 hours of dosing on that day.

APPENDIX 1 (CONTINUED)

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL MATERNAL OBSERVATIONS

300 MG/KG

RAT	Ataxia	Dyspnea	Rales	Vocal-ization	Nasal Congestion	Fluid Around Nose	Sali-vation
2010			BCDEfg		GJ		j
2011		A	BcHIj		AeGj		
2015			CDEfJ	C	G		
2018			ABEfIJ		aDegi	a	a
2021			D		dEfgIJ	dg	dg
2022							
2023			EfgK			e	e
2027			ef		e		
2030			ABch	B	cIK	ch	ach
2033			c				
2034			EfHi	E			
2039			J	j			
2045							
2048			AbHi	c	dFG	a	ac
2057			G				

	Other Observations	
2010	Alopecia	DEFGHIJKN136
2023	Alopecia	136
2033	Alopecia	N136

Note: Each character indicates the day the condition was observed:
A = GD6, B = GD7, C = GD8... 1 = PD1, 3 = PD3, 6 = PD6.
A lowercase letter indicates the condition was observed only within the first 4 hours of dosing on that day.

APPENDIX 1 (CONTINUED)

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL MATERNAL OBSERVATIONS

400 MG/KG

RAT	Ataxia	Dyspnea	Rales	Vocal-ization	Nasal Congestion	Fluid Around Nose	Sali-vation
2008	i		AfJ	a	b		
2009			CdEfH	C	G	h	h
2017			ABCdGHJ	ag	DEfH	h	h
2020			eGhN		BEj	eh	eh
2025			BCI		Df	a	a
2029		A	AH		BcEGhijN	aj	j
2031			EFGHIJ	abcj	A		e
2035			CGHiJK16		efHN	j	j
2038			GHi	g	N	j	j
2041			a	AB			
2046			ADJK		beI	de	ade
2050	i		EFhIjK	E	eg		
2052			ABCDEIJ		eFg	ae	ae
2053			CDehj	C		j	j
2054			A	a	E		

Other Observations

2050	Red vaginal discharge	i
2054	Mass: firm, not movable, right hind leg	CDEFGHIJKN136

Note: Each character indicates the day the condition was observed:
A = GD6, B = GD7, C = GD8... 1 = PD1, 3 = PD3, 6 = PD6.
A lowercase letter indicates the condition was observed only within the first 4 hours of dosing on that day.

APPENDIX 2

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RAT

INDIVIDUAL BODY WEIGHTS (g)

CONTROL

	Gestation Day						
Rat	3	6	8	10	13	16	20
2012	276	298	308	319	343	383	454
2013	261	279	282	298	327	354	429
2014	261	286	295	305	328	348	408
2016a	249	278	288	294	282	282	283
2019	269	280	293	296	318	351	404
2024	266	279	293	303	325	342	402
2026	253	273	280	290	322	355	425
2028	256	276	274	287	308	333	404
2032	268	283	293	302	312	353	405
2036	258	278	284	296	313	340	394
2037	261	284	293	304	324	350	408
2040	244	271	291	307	310	340	393
2042	252	273	284	296	319	343	412
2043a	242	262	254	265	281	271	278
2044	247	265	276	285	311	348	405
2047a	256	272	274	281	278	279	291
2049	255	277	288	305	323	364	435
2051	255	263	276	299	314	349	408
2055	250	280	285	297	324	348	402
2056	259	285	302	316	350	376	455
MEAN	258.3	278.2	288.1	300.3	321.8	351.6	414.3
S.E.	2.0	2.0	2.3	2.2	2.7	3.1	4.6

N = 17

a Not pregnant

APPENDIX 2 (CONTINUED)

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL BODY WEIGHTS (g)

300 MG/KG

	Gestation Day						
Rat	3	6	8	10	13	16	20
2010a	243	253	234	249	266	266	274
2011a	256	281	262	289	297	299	292
2015	257	285	290	278	301	334	389
2018	253	272	250	279	306	315	391
2021	270	296	302	279	309	343	406
2022	261	272	281	297	323	349	407
2023	250	267	273	287	298	320	398
2027a	245	260	264	267	275	266	270
2030	246	257	237	228	258	286	355
2033	263	287	300	311	331	352	389
2034	251	279	279	288	303	317	374
2039	265	282	294	304	313	335	392
2045a	256	292	300	309	308	308	318
2048	268	284	264	289	306	337	402
2057	272	300	306	326	348	376	432
MEAN	259.6	280.1	279.6	287.8	308.7	333.1	394.1
S.E.	2.7	3.8	6.7	7.5	6.8	7.1	5.9

N = 11

a Not pregnant

APPENDIX 2 (CONTINUED)

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL BODY WEIGHTS (g)

400 MG/KG

	Gestation Day						
Rat	3	6	8	10	13	16	20
2008a	246	258	249	257	274	238	257
2009	274	283	304	302	319	364	422
2017	247	272	250	274	297	304	368
2020	272	295	303	314	326	350	416
2025	253	279	264	295	304	349	406
2029	257	281	243	265	274	292	335
2031	248	269	276	283	292	322	388
2035	255	278	254	288	291	289	362
2038a	242	268	282	286	284	280	274
2041	251	278	273	289	320	345	420
2046	260	291	272	296	323	351	414
2050	265	295	295	309	325	282	343
2052	266	291	255	287	303	331	397
2053	263	292	297	279	322	334	386
2054	256	284	268	288	320	347	411
MEAN	259.0	283.7	273.4	289.9	308.9	327.7	389.8
S.E.	2.4	2.4	5.7	3.8	4.6	7.6	8.2

N = 13

a Not pregnant

APPENDIX 3

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL PARTURITION AND POSTNATAL DATA

CONTROL

			Day 1				Day 3		Day 6				
	Gestation Length		Number of Pups		Weight(g)		Number of Pups		Number of Pups		Weight(g)		
Rat	Days	Rank[r]	Live	Dead	Litter	Pup	Live	Dead	Live	Dead	Litter	Pup	Implants
2012	21	3	12	0	88.7	7.4	12	0	12	0	167.7	14.0	12
2013	21	29.5	13	0	81.3	6.3	13	0	12	0	145.6	12.1	13
2014	21	37	8	0	55.0	6.9	8	0	8	0	118.2	14.8	9
2016a	.	.	.	.	.	.	.	.	.	.	.	.	.
2019	21	29.5	13	1	79.2	6.1	13	0	13	0	163.7	12.6	14
2024	21	10	9	0	67.5	7.5	9	0	9	0	131.3	14.6	13
2026	21	18.5	11	0	90.4	8.2	11	0	11	0	175.1	15.9	12
2028	21	34	12	0	84.6	7.1	12	0	12	0	181.4	15.1	13
2032	22	40	11	0	73.3	6.7	11	0	11	0	146.1	13.3	14
2036	21	3	12	0	89.6	7.5	12	0	12	0	173.5	14.5	14
2037	21	29.5	11	0	78.7	7.2	11	0	11	0	164.0	14.9	11
2040	21	12.5	13	0	100.3	7.7	13	0	13	0	173.7	13.4	14
2042	21	3	13	0	93.1	7.2	13	0	13	0	178.9	13.8	13
2043a	.	.	.	.	.	.	.	.	.	.	.	.	.
2044	21	12.5	11	0	85.9	7.8	11	0	11	0	173.9	15.8	14
2047a	.	.	.	.	.	.	.	.	.	.	.	.	.
2049	21	3	12	0	94.3	7.9	12	0	12	0	187.3	15.6	13
2051	21	23	14	0	88.8	6.3	14	0	14	0	174.9	12.5	15
2055	21	18.5	11	0	75.6	6.9	11	0	11	0	149.6	13.6	12
2056	21	23	14	0	91.0	6.5	14	0	14	0	184.4	13.2	15
MEAN		19.4	11.8			7.1	11.8		11.7			14.1	13.0
S.E.		3.1	0.4			0.1	0.4		0.4			0.3	0.4
N		17	17			17	17		17			17	17

a Not pregnant
r Ranking based on the observed time and stage of parturition.

APPENDIX 3 (CONTINUED)

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL PARTURITION AND POSTNATAL DATA

300 MG/KG

			Day 1				Day 3		Day 6				
	Gestation Length		Number of Pups		Weight(g)		Number of Pups		Number of Pups		Weight(g)		
Rat	Days	Rank[r]	Live	Dead	Litter	Pup	Live	Dead	Live	Dead	Litter	Pup	Implants
2010a	.	.	.	.	.	.	.	.	.	.	.	.	.
2011a	.	.	.	.	.	.	.	.	.	.	.	.	.
2015	21	15	6	0	50.6	8.4	6	0	6	0	104.1	17.4	11
2018	21	16.5	13	0	84.2	6.5	13	0	13	0	172.3	13.3	14
2021	21	23	11	0	86.7	7.9	11	0	11	0	172.4	15.7	14
2022	21	23	13	0	86.8	6.7	13	0	13	0	181.1	13.9	14
2023	22	39	14	0	95.3	6.8	14	0	14	0	161.3	11.5	14
2027a	.	.	.	.	.	.	.	.	.	.	.	.	.
2030	21	23	14	0	85.3	6.1	14	0	14	0	179.7	12.8	14
2033	21	36	4	0	27.5	6.9	4	0	0	0	.	.	4
2034	21	8.5	7	0	58.2	8.3	7	0	7	0	132.7	19.0	11
2039	21	3	9	0	69.7	7.7	9	0	9	0	150.6	16.7	10
2045a	.	.	.	.	.	.	.	.	.	.	.	.	.
2048	21	12.5	9	0	75.8	8.4	9	0	9	0	142.4	15.8	10
2057	21	12.5	9	0	80.4	8.9	9	0	9	0	162.9	18.1	11
MEAN		19.3	9.9			7.5	9.9		9.5			15.4	11.5
S.E.		3.3	1.0			0.3	1.0		1.3			0.8	0.9
N		11	11			11	11		11			10	11

a Not pregnant
r Ranking based on the observed time and stage of parturition.

APPENDIX 3 (CONTINUED)

2-METHYLHEXANOIC ACID
DEVELOPMENTAL TOXICITY TESTING IN SPRAGUE-DAWLEY RATS

INDIVIDUAL PARTURITION AND POSTNATAL DATA

400 MG/KG

			Day 1				Day 3		Day 6				
	Gestation Length		Number of Pups		Weight(g)		Number of Pups		Number of Pups		Weight(g)		
Rat	Days	Rank[r]	Live	Dead	Litter	Pup	Live	Dead	Live	Dead	Litter	Pup	Implants
2008a	.	.	.	.	.	.	.	.	.	.	.	.	.
2009	21	6.5	10	0	73.2	7.3	10	0	10	0	144.3	14.4	14
2017	21	16.5	14	0	84.2	6.0	14	0	14	0	154.4	11.0	14
2020[b]	21	34	11	1	70.7	6.4	11	0	.	.	.	.	13
2025	21	8.5	13	0	98.9	7.6	12	0	12	0	151.4	12.6	14
2029	21	29.5	7	0	51.9	7.4	7	0	7	0	111.0	15.9	13
2031	21	29.5	12	0	80.4	6.7	12	0	12	0	162.0	13.5	14
2035	21	23	11	0	73.2	6.7	11	0	11	0	154.2	14.0	13
2038a	.	.	.	.	.	.	.	.	.	.	.	.	.
2041	21	34	14	0	97.4	7.0	14	0	14	0	179.5	12.8	15
2046	21	23	11	0	84.7	7.7	11	0	11	0	173.7	15.8	13
2050	22	38	11	0	67.3	6.1	11	0	11	0	123.5	11.2	12
2052	21	6.5	10	0	73.8	7.4	10	0	10	0	132.3	13.2	12
2053	22	41	7	0	50.9	7.3	7	0	7	0	117.9	16.8	7
2054	21	29.5	9	0	63.0	7.0	9	0	9	0	152.2	16.9	10
MEAN		24.6	10.8			7.0	10.7		10.7			14.0	12.6
S.E.		3.3	0.6			0.2	0.6		0.7			0.6	0.6
N		13	13			13	13		12			12	13

a Not pregnant
b Day-6 data excluded from calculations due to malfunctioning water spigot.
r Ranking based on the observed time and stage of parturition.

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16. ABSTRACT

As part of an investigation of the developmental effects and structure-activity relationships of aliphatic acids, 2-methylhexanoic acid was administered by gavage to Sprague-Dawley rats on gestation days 6-15 at doses of 0, 300, and 400 mg/kg/day. The dams were allowed to deliver and their litters were examined through postnatal day 6. Pups that were found dead were examined for soft-tissue alterations. On day 6, two survivors per litter were preserved for skeletal examination. Maternal toxicity was demonstrated at both 300 and 400 mg/kg by weight loss and altered respiration (rales, dyspnea). In spite of the maternal toxicity present, there were no clear toxic effects on development; litter size, pre- and postnatal viability, and pup weights were unaffected by treatment. Skeletal examinations of selected pups yielded inconclusive data; a slight increase in the incidence of lumbar ribs was present a 400 mg/kg, but was not clearly attributable to treatment.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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