VELSICOL CHEMICAL CORPORATION

10400 W. HIGGINS RD., SUITE 600
ROSEMONT, IL 60018-3713
(708) 298-9000
FAX: (708) 298-9014

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November 10, 1994

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INIT 11/10/94

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VIA MESSENGER

Document Control Officer (7407)
Room E-G99
Attention TSCA 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Re: PMN P-90-549 (CASRN 131298-44-7); Interim Report from Study Performed Under 5(e) Consent Order

Dear Sir or Madam:

Velsicol Chemical Corporation submits the following report for your information and in compliance with section 8(e) of TSCA, if necessary:

Interim report for the twenty-eight day oral toxicity study in the rat with isodecyl benzoate. (Individual animal data also appended.)

In this study, various effects observed during the functional observational battery (FOB) may indicate behavioral responses to some aspect of general malaise from high doses, or could be indications of a mild effect on nervous system competence. In the FOB, increased foot splay and palpebral closure, as well as decreased rearing and activity were seen in the high dose groups, primarily in the females.

The interim report also notes that the control and pre-dose incidence of "slight tremor" (strain is Sprague-Dawley Clr: CD BR VAF PLUS) may have increased in frequency in the high-dose groups, and that the intensity, in one or two high-dose females, was increased from slight to moderate. This raises a question of whether the information is subject to section 8(e) reporting requirements. Due to the incidence of effects in the control and pre-dose groups, we are not able to reach a definitive conclusion about the significance of these effects or their relation to dosage. When the final report of this study becomes available, we will promptly submit it to the Agency.

12/15/94

Printed on Recycled Pap

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If you have any questions concerning this submission, please call me at 708-635-3454.

Sincerely,

John M. Bergman /PCD

John M. Bergman
Manager, Regulatory Affairs

Attachment

cc: R. Allison,
OPPT/CCD/NCB

HRC

INTERIM REPORT FOR THE TWENTY-EIGHT DAY ORAL TOXICITY STUDY IN THE RAT WITH ISODECYL BENZOATE

HRC Schedule No.: VCL/206

The following report contains interim data (unaudited) generated up to the terminal sacrifice in the above study.

Treatment commenced on 25 August 1994 with a total of 64 rats allocated to groups as follows:

Group	Treatment	Dosage level (mg/kg/day)	No. of rats ♂	No. of rats ♀	Rat numbers ♂	Rat numbers ♀
1	Control, Corn oil	-	8	8	1-8	33-40
2	Isodecylbenzoate	15	8	8	9-16	41-48
3	Isodecylbenzoate	150	8	8	17-24	49-56
4	Isodecylbenzoate	1000	8	8	25-32	57-64

The appropriate concentration of the test substance, formulated as 20, 3.0 and 0.3 % w/v suspensions in corn oil was administered by oral gavage once daily, to each rat of Groups 2 to 4 at a dose volume of 5 ml/kg/day. Control animals received the vehicle (corn oil) alone at the same dose volume (5 ml/kg/day).

Bodyweights and food consumption were recorded on a weekly basis and visual assessment of water consumption was maintained throughout the treatment period. A functional observational battery was performed on all animals prior to study commencement and subsequently during Weeks 2 and 4. A draft report for this investigation is attached (Appendix 6).

Blood was withdrawn from five males and five females (lowest animal numbers) from each group during Week 4 (Day 27) for haematological and biochemical investigations.

Following 29 consecutive days of treatment, five males and five females (lowest animal numbers) from each group were killed (Day 30, 23 September 1994) and underwent a macroscopic *post mortem* examination. Weights were recorded for specified organs and relevant fixed tissue samples were prepared for microscopic examination. The remaining animals from each group were killed by cervical dislocation and discarded with no further investigations performed.

HRC

ORGAN WEIGHTS (Table 5, Appendix 5)

For rats of both sexes treated at 1000 mg/kg/day higher than control liver weight (bodyweight-adjusted) was recorded.

Kidney weights (bodyweight-adjusted) were statistically significantly higher than control for males treated at 1000 mg/kg/day.

The toxicological importance of the above differences in kidney and liver weight may become clearer following histopathological examination.

Statistically significantly higher than control ovary (bodyweight-adjusted) and spleen weights were recorded for high dosage females. However, individual values overlapped to a large extent and these were not considered to be treatment-related effects.

There were no other statistically significant differences between treated and control rats.

MACROSCOPIC PATHOLOGY (Table 6)

The macroscopic *post-mortem* examination performed at termination revealed enlarged liver for four males and two females receiving 1000 mg/kg/day.

There were no other macroscopic changes observed that were considered to be related to treatment.

MICROSCOPIC PATHOLOGY

To be included in the draft report

HRC

Lymphocyte count, and consequently total white blood cell count, was statistically significantly lower than control for male rats treated at 1000 mg/kg/day. Statistically significantly lower total white blood cell count was also recorded for males receiving 150 mg/kg/day. Individual values overlapped between the groups and these differences from control were considered to be attributable to chance.

Statistically significantly lower than control mean corpuscular volume (MCV) was recorded for females receiving 1000 mg/kg/day. There was considerable overlap between the groups and the majority of values were within the background range for rats of this age and strain, this minor difference from control was not considered to be related to treatment.

The occurrence of slight polychromasia and/or slight anisocytosis is not uncommon in young laboratory rats and at the incidence seen in this study was not considered to be related to treatment with the test material.

BIOCHEMISTRY (Table 4, Appendix 4)

The following findings were considered to be of note, however, their relationship to treatment remains unclear at this stage.

Glucose levels were statistically significantly lower than control for males and females receiving 1000 mg/kg/day and for females treated at 150 or 15 mg/kg/day. All individual values for rats from the intermediate and low dosage groups were within the expected range for rats of this age and strain, in the absence of a dosage-relationship a treatment-related effect at 150 or 15 mg/kg/day was not suspected.

Statistically significantly higher than control albumin was recorded for males receiving 1000 or 150 mg/kg/day, the resulting increase in A/G ratio also attained statistical significance for these groups though total protein remained unaffected. For females receiving 1000 mg/kg/day statistically significantly higher than control total protein was recorded.

Creatinine levels for high dosage males were statistically significantly higher than for control.

In addition, sodium levels for males treated at 1000 mg/kg/day were statistically significantly higher than control.

For females receiving 1000 mg/kg/day, glutamic-pyruvic transaminase (GPT) was statistically significantly lower than control. However, individual values overlapped between the groups and the majority were within the expected range for rats of this age and strain. This difference was considered to be attributable to natural variation.

There were no other statistically significant differences between treated and control rats.

HRC

RESULTS

CLINICAL SIGNS

There were no mortalities.

Appearance indicative of a slight lack of grooming was noted during Week 4 for all males and females receiving 1000 mg/kg/day.

An increase in salivation following dosing, commonly associated with wet fur, was seen for all high dosage animals. These are common findings in rat orally-dosed studies and are considered to be related to the palatability of the test material and not toxicologically important.

There were no clinical findings for rats treated at 150 or 15 mg/kg/day or for controls.

BODYWEIGHT GAINS (Figure 1, Table 1, Appendix 1)

There were no statistically significant differences in bodyweight gain between treated and control rats.

FOOD CONSUMPTION (Figure 2, Table 2, Appendix 2)

There were no differences in food consumption that were considered to be related to treatment.

WATER CONSUMPTION

Visual assessment of water consumption revealed no differences considered to be related to treatment.

FUNCTIONAL OBSERVATIONAL BATTERY (Appendix 6)

The results of the functional observational batery are presented in Appendix 6.

HAEMATOLOGY (Table 3, Appendix 3)

There were no differences from control that were considered to be related to treatment with Isodecyl benzoate.

FIGURE 1

VCL/206

Bodyweights – group mean values

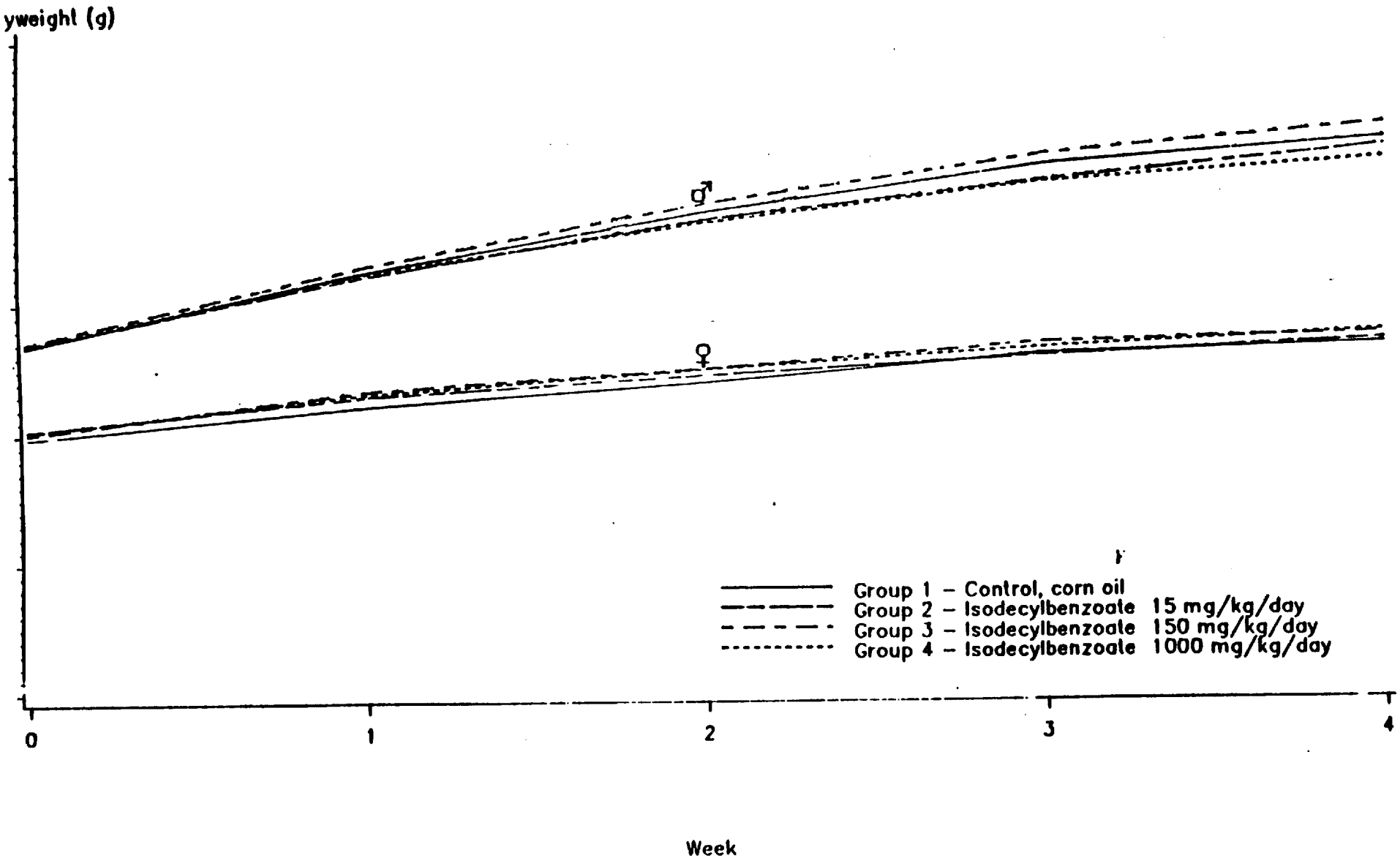
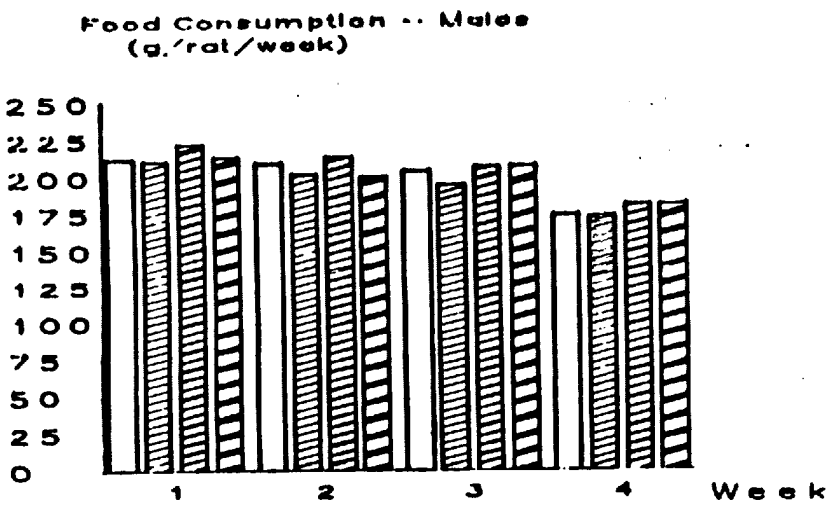
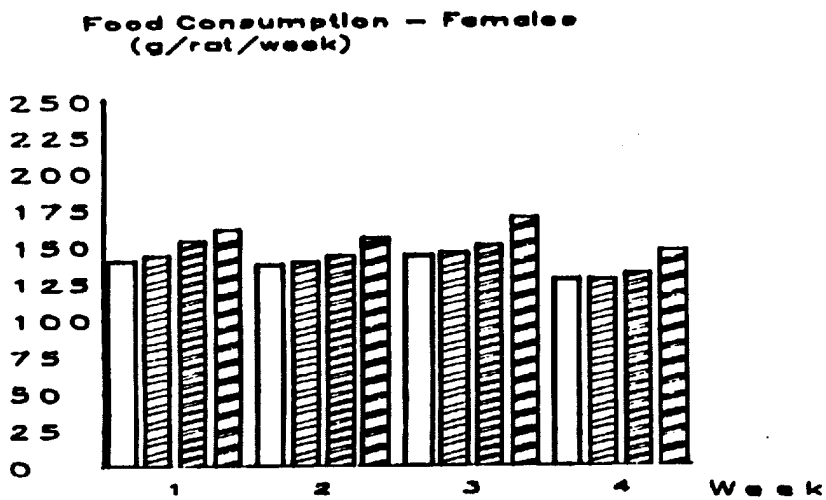


FIGURE 2

Food Consumption – group mean values





Group 1 -- Control, corn oil
Group 2 – Isodecylbenzoate 15 mg./kg/da
Group 3 -- Isodecylbenzoate 150 mg./kg/d
Group 4 -- Isodecylbenzoate 1000 mg/kg./

TABLE 1

Bodyweights - group mean values (g)

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 15	3♂ 150	4♂ 1000	1♀ Control	2♀ 15	3♀ 150	4♀ 1000
Dosing								
0	268	269	271	269	198	204	201	203
1	324	320	328	323	221	228	233	230
2	369	363	375	360	238	243	248	248
3	404	392	411	390	259	257	267	263
4	422	416	433	407	266	268	273	275
Gain Wk 0-4	154	147	162	138	68	64	72	72
% of control	-	95	105	90	-	94	106	106

No statistical significance (P>0.05) for bodyweight gain

TABLE 2

Food consumption - group mean values (g/rat/week)

Week	Group and dosage (mg/kg/day)							
	1♂ Control	2♂ 15	3♂ 150	4♂ 1000	1♀ Control	2♀ 15	3♀ 150	4♀ 1000
Dosing								
1	212	210	222	214	140	144	154	161
2	209	202	213	200	138	139	144	156
3	204	195	206	207	144	145	150	169
4	174	172	181	180	127	128	132	147
Cumulative consumption	799	779	822	801	549	556	580	633
% of control	-	97	103	100	-	101	106	115

No statistical significance (P>0.05) for cumulative consumption

TABLE 3

Haematology - group mean values

Week 4

Group/ dosage mg/kg/day	PCV %	Hb g/dl	RBC x106/ mm3	MCHC %	MCV fl	WBC + Diff x103/mm3 Total	N	L	E	B	M	Plts x103/ mm3	TT s
1♂ Control	56	15.3	6.9	27.4	81	13.8	LT 2.91	10.45	0.12	NA 0.00	0.28	868	21
2♂ 15	56	15.4	7.2	27.3	79	11.8	2.04	9.52	0.06	0.00	0.18	672	22
3♂ 150	57	15.2	7.2	26.9	79	* 9.2	1.31	7.79	0.07	0.00	0.05	1014	21
4♂ 1000	56	15.4	7.0	27.5	81	** 8.0	1.41	** 6.47	0.02	0.00	0.07	762	22
1♀ Control	56	15.1	6.9	26.8	82	6.8	1.17	5.61	0.02	NA 0.00	0.02	919	20
2♀ 15	58	15.5	7.1	26.9	81	8.3	1.07	7.20	0.00	0.00	0.06	865	19
3♀ 150	56	14.9	6.9	26.6	81	6.4	1.37	4.89	0.14	0.00	0.01	922	19
4♀ 1000	55	14.7	6.8	26.9	* 80	7.0	1.58	5.35	0.02	0.00	0.05	887	19

* P<0.05
** P<0.01
LT Data log-transformed
NA No analysis necessary; all values the same

TABLE 4

Biochemistry - group mean values

Week 4

Group/ dosage mg/kg/day	Glu-cose mg/dl	Protein g/dl Total	Alb	Glob	A/G	Urea Nitr mg/dl	Creat-inine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
1♂ Control	121	6.6	2.7	3.9	0.69	10	0.5	383	25	56	F 0.1	144	3.6	5.7	4.9	98	65
2♂ 15	135	6.5	2.7	3.8	0.72	10	0.4	348	29	51	0.1	144	3.9	5.6	4.7	99	67
3♂ 150	112	6.6	* 2.9	3.7	* 0.77	10	0.5	342	28	53	0.1	146	3.6	5.6	4.8	99	69
4♂ 1000	** 89	6.6	** 3.0	3.6	** 0.82	10	* 0.6	374	25	52	0.1	* 147	3.7	5.5	5.0	100	61
1♀ Control	126	6.7	3.0	3.7	0.81	12	0.5	236	27	49	F 0.1	145	3.6	5.7	4.0	101	73
2♀ 15	* 112	6.7	3.0	3.7	0.83	13	0.5	173	25	53	0.1	145	3.5	5.7	4.3	101	74
3♀ 150	* 118	6.9	3.0	3.9	0.78	12	0.5	218	23	51	0.1	144	3.4	5.6	4.0	100	69
4♀ 1000	** 102	* 7.2	3.1	4.1	0.78	14	0.6	232	* 21	52	0.1	144	3.4	5.7	4.3	100	87

* $P<0.05$
** $P<0.01$
F Frequency analysis

VCL/206

TABLE 5

Organ weights - group mean values

Week 5

Group/ dosage mg/kg/day	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g	Prostate g
1♂ Control	430	2.18	22.3	0.93	3.43	60.3	1.781	1.809	1.14	0.525	0.495	0.81
2♂ 15	410	2.09	20.7	0.87	3.25	58.5	1.660	1.666	1.08	0.496	0.497	0.86
3♂ 150	431	2.07	20.0	0.84	3.49	57.5	1.790	1.761	1.08	0.489	0.487	0.75
4♂ 1000	418	2.13	27.8	0.84	3.76	64.2	1.761	1.758	0.88	0.536	0.496	0.84
Adjusted means												
1♂		-	21.8	0.91	3.38	-	1.772	1.801	-	-	0.493	-
2♂		-	21.2	0.92	3.34	-	1.676	1.679	-	-	0.502	-
3♂		-	19.4	0.81	3.43	-	1.778	1.752	-	-	0.484	-
4♂		-	** 28.1	0.86	* 3.79	-	1.766	1.763	-	-	0.498	-

* P<0.05
** P<0.01

TABLE 5

(Organ weights - continued)

Week 5

Group/ dosage mg/kg/day	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Control	266	1.93	11.9	0.61	2.38	74.4	114.6
2♀ 15	263	1.96	11.6	0.67	2.21	74.9	109.2
3♀ 150	271	1.92	12.3	0.66	2.43	81.0	113.6
4♀ 1000	274	1.95	18.7	0.73*	2.65	80.1	96.8
Adjusted means							
1♀		-	12.0	-	-	-	116.2
2♀		-	11.8	-	-	-	112.4
3♀		-	12.2	-	-	-	112.1
4♀		-	18.5**	-	-	-	93.5*

* P<0.05
** P<0.01

STUDY NO: VCL206 TITLE: TOXICITY TO RATS WITH ISODECYL BENZOATE

P A T H O L O G Y - INTERGROUP COMPARISON OF MACROSCOPIC FINDINGS INCIDENCE

TITLE: TOXICITY TO RATS WITH ISODECYL BENZOATE

REMOVAL REASON: TERMINAL	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	8	8	8	8	8	8	8	8
ANIMALS COMPLETED	5	5	5	5	5	5	5	5
LYMPH NODES - CERVICAL								
Enlarged	2	3	3	2	0	0	2	2
LIVER								
Median cleft, pale subcapsular area/s.	0	0	0	1	0	0	0	0
Enlarged	0	0	0	4	0	0	0	2
SPLEEN								
Clear fluid-filled cyst/s	0	0	0	0	1	0	0	0
Capsule thickened	0	0	0	0	1	0	0	1
STOMACH ANTRUM MUCOSA								
White nodule/s	1	0	0	0	1	0	2	1
KIDNEYS								
Increased pelvic dilatation	1	0	0	0	0	0	0	0
OVARIES								
Clear fluid-filled cyst	0	0	0	0	0	0	0	1
UTERUS								
Fluid distension	0	0	0	0	0	1	1	0
Thickened	0	0	0	0	0	0	1	0
CERVIX								
Swollen	0	0	0	0	0	0	1	0

APPENDIX 1

Bodyweights - individual values (g)

Group 1♂
Control

Cage number	Animal number	Week 0	1	2	3	4
1	1	274	349	409	469	499
	2	265	303	335	366	376
	3	263	324	362	380	393
	4	278	339	396	444	460
2	5	273	332	383	426	444
	6	261	303	339	361	380
	7	260	306	347	371	389
	8	272	334	379	412	435

Group 2♂
15 mg/kg/day

Cage number	Animal number	Week 0	1	2	3	4
3	9	248	282	319	339	367
	10	275	331	373	408	426
	11	275	330	387	420	454
	12	272	330	375	420	446
4	13	260	314	351	375	382
	14	275	320	354	370	400
	15	264	318	362	393	418
	16	282	337	381	408	436

Group 3♂
150 mg/kg/day

Cage number	Animal number	Week 0	1	2	3	4
5	17	269	322	369	399	417
	18	290	352	403	451	472
	19	269	327	372	404	422
	20	260	304	344	379	402
6	21	279	345	402	445	473
	22	262	319	358	387	407
	23	256	325	382	422	453
	24	281	329	370	402	417

Group 4♂
1000 mg/kg/day

Cage number	Animal number	Week 0	1	2	3	4
7	25	269	322	358	379	381
	26	261	306	333	347	357
	27	277	346	391	430	450
	28	269	315	352	378	398
8	29	283	357	414	463	490
	30	271	316	353	379	399
	31	260	311	347	381	390
	32	264	307	335	366	389

Group 1♀
Control

Cage number	Animal number	Week 0	1	2	3	4
9	33	192	219	228	254	259
	34	201	218	246	269	273
	35	207	232	248	264	269
	36	204	231	238	262	268
10	37	197	220	241	260	269
	38	197	216	238	255	257
	39	188	209	227	248	259
	40	195	220	238	256	270

Group 2♀
15 mg/kg/day

Cage number	Animal number	Week 0	1	2	3	4
11	41	199	224	241	249	266
	42	204	229	248	257	273
	43	209	238	256	269	277
	44	202	227	233	251	253
12	45	206	229	240	260	276
	46	209	237	254	268	284
	47	183	202	218	225	226
	48	217	240	256	277	289

Group 3♀
150 mg/kg/day

Cage number	Animal number	Week 0	1	2	3	4
13	49	202	223	242	262	273
	50	220	248	267	289	302
	51	200	232	251	259	262
	52	195	215	225	246	251
14	53	196	230	251	269	268
	54	207	250	264	290	294
	55	186	230	232	245	247
	56	203	232	253	277	287

Group 4♀
1000 mg/kg/day

Cage number	Animal number	Week 0	1	2	3	4
15	57	212	244	266	281	292
	58	192	213	236	248	248
	59	204	226	237	261	271
	60	204	230	240	263	273
16	61	212	241	249	274	292
	62	201	234	258	269	276
	63	191	222	238	245	265
	64	208	231	257	266	280

APPENDIX 2

Food consumption - cage mean values (g/rat/week)

Group 1♂
Control

Week	Cage 1	Cage 2
Dosing		
1	211	213
2	213	205
3	212	197
4	177	170

Group 2♂
15 mg/kg/day

Week	Cage 3	Cage 4
Dosing		
1	207	214
2	203	201
3	200	189
4	173	171

Group 3♂
150 mg/kg/day

Week	Cage 5	Cage 6
Dosing		
1	215	230
2	205	221
3	193	220
4	174	187

Group 4♂
1000 mg/kg/day

Week	Cage 7	Cage 8
Dosing		
1	214	213
2	193	206
3	197	217
4	169	191

Group 1♀
Control

Week	Cage 9	Cage 10
Dosing		
1	142	138
2	135	140
3	141	146
4	126	129

Group 2♀
15 mg/kg/day

Week	Cage 11	Cage 12
Dosing		
1	138	149
2	133	146
3	142	149
4	121	134

Group 3♀
150 mg/kg/day

Week	Cage 13	Cage 14
Dosing		
1	148	159
2	139	148
3	145	156
4	129	134

Group 4♀
1000 mg/kg/day

Week	Cage 15	Cage 16
Dosing		
1	154	167
2	148	164
3	162	175
4	136	158

APPENDIX 3

Haematology - individual values

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	PCV %	Hb g/dl	RBC x106/ mm3	MCHC %	MCV fl	WBC + Diff x103/mm3 Total	N	L	E	B	M	Plts x103/ mm3	TT s
1♂ Control	1 PA	51	14.6	6.1	28.6	84	14.8	2.66	11.40	0.30	0.00	0.44	975	21
	2	57	15.1	7.2	26.5	79	8.3	1.58	6.47	0.08	0.00	0.17	937	22
	3 A	59	16.0	7.7	27.1	77	11.8	1.65	9.68	0.24	0.00	0.24	735	20
	4 PA	54	15.2	6.6	28.1	82	20.0	7.00	12.60	0.00	0.00	0.40	847	21
	5	58	15.6	7.0	26.9	83	13.9	1.67	12.09	0.00	0.00	0.14	844	23
	Mean	56	15.3	6.9	27.4	81	13.8	2.91	10.45	0.12	0.00	0.28	868	21
	SD	3.3	0.53	0.61	0.88	2.9	4.29	2.328	2.482	0.139	0.000	0.135	93.4	1.1
2♂ 15	9	57	15.2	7.3	26.7	78	12.1	1.33	10.65	0.12	0.00	0.00	668	23
	10	57	15.4	7.2	27.0	79	9.1	2.00	7.01	0.00	0.00	0.09	685	25
	11	55	15.2	6.7	27.6	82	17.2	3.96	12.56	0.00	0.00	0.69	741	23
	12	56	15.4	7.3	27.5	77	12.3	2.09	10.09	0.00	0.00	0.12	566	20
	13	57	15.9	7.4	27.9	77	8.3	0.83	7.30	0.17	0.00	0.00	702	21
	Mean	56	15.4	7.2	27.3	79	11.8	2.04	9.52	0.06	0.00	0.18	672	22
	SD	0.9	0.29	0.28	0.48	2.1	3.50	1.189	2.349	0.081	0.000	0.290	65.3	1.9
3♂ 150	17 A	56	15.7	6.8	28.0	82	8.2	1.80	6.31	0.08	0.00	0.00	956	20
	18	CTD	CTD	CTD	NR	NR	CTD	CTD	CTD	CTD	CTD	CTD	CTD	21
	19 PA	56	15.0	7.2	26.8	78	10.1	1.31	8.59	0.10	0.00	0.10	829	21
	20	58	15.5	7.3	26.7	79	10.9	1.20	9.70	0.00	0.00	0.00	909	21
	21	56	14.6	7.3	26.1	77	7.6	0.91	6.54	0.08	0.00	0.08	1360	23
	Mean	57	15.2	7.2	26.9	79	9.2	1.31	7.79	0.07	0.00	0.05	1014	21
	SD	1.0	0.50	0.24	0.80	2.2	1.56	0.371	1.637	0.044	0.000	0.053	236.9	1.3
4♂ 1000	25 P	56	15.6	7.0	27.9	80	6.6	1.39	5.15	0.00	0.00	0.07	844	23
	26	57	14.9	7.4	26.1	77	6.4	1.28	4.93	0.00	0.00	0.19	710	23
	27	58	16.2	7.0	27.9	83	9.4	2.07	7.24	0.00	0.00	0.09	730	23
	28	56	15.4	7.1	27.5	79	8.2	1.56	6.56	0.08	0.00	0.00	768	20
	29 PA	53	15.0	6.3	28.3	84	9.2	0.74	8.46	0.00	0.00	0.00	760	20
	Mean	56	15.4	7.0	27.5	81	8.0	1.41	6.47	0.02	0.00	0.07	762	22
	SD	1.9	0.52	0.40	0.85	2.9	1.41	0.481	1.473	0.036	0.000	0.078	51.2	1.6

SD Standard deviation
CTD Clotted Sample
NR Not recorded
P Slight polychromasia
A Slight anisocytosis

APPENDIX 3

(Haematology - continued)

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	PCV %	Hb g/dl	RBC x106/ mm3	MCHC %	MCV fl	WBC + Diff x103/mm3 Total	N	L	E	B	M	Plts x103/ mm3	TT s
1♀ Control	33 P	55	15.2	6.8	27.6	81	6.8	0.54	6.26	0.00	0.00	0.00	819	21
	34 PA	54	14.4	6.7	26.7	81	6.8	1.09	5.71	0.00	0.00	0.00	1002	19
	35	59	15.2	7.1	25.8	83	8.3	1.83	6.39	0.08	0.00	0.00	891	21
	36	56	15.4	6.7	27.5	84	6.3	0.88	5.42	0.00	0.00	0.00	1082	20
	37 PA	58	15.4	7.0	26.6	83	5.9	1.53	4.25	0.00	0.00	0.12	801	18
	Mean	56	15.1	6.9	26.8	82	6.8	1.17	5.61	0.02	0.00	0.02	919	20
	SD	2.1	0.41	0.18	0.74	1.3	0.91	0.513	0.855	0.036	0.000	0.054	120.6	1.3
2♀ 15	41	59	15.6	7.1	26.4	83	10.8	1.08	9.50	0.00	0.00	0.22	940	19
	42	59	16.5	7.2	28.0	82	8.6	1.38	7.22	0.00	0.00	0.00	817	20
	43	57	15.2	6.9	26.7	83	5.8	0.81	4.99	0.00	0.00	0.00	778	19
	44	59	15.4	7.4	26.1	80	8.5	1.11	7.40	0.00	0.00	0.00	792	19
	45	54	14.8	6.8	27.4	79	7.9	0.95	6.87	0.00	0.00	0.08	1000	18
	Mean	58	15.5	7.1	26.9	81	8.3	1.07	7.20	0.00	0.00	0.06	865	19
	SD	2.2	0.63	0.24	0.77	1.8	1.79	0.212	1.606	0.000	0.000	0.096	98.8	0.7
3♀ 150	49 A	54	14.4	6.6	26.7	82	7.7	2.77	4.70	0.23	0.00	0.00	837	19
	50	58	15.4	7.1	26.6	82	7.3	1.24	5.91	0.15	0.00	0.00	929	20
	51	57	15.0	7.0	26.3	81	6.6	1.25	5.28	0.07	0.00	0.00	799	19
	52	56	15.0	6.9	26.8	81	4.5	1.22	3.02	0.23	0.00	0.05	977	19
	53	55	14.5	6.8	26.4	81	5.9	0.35	5.55	0.00	0.00	0.00	1070	CTD
	Mean	56	14.9	6.9	26.6	81	6.4	1.37	4.89	0.14	0.00	0.01	922	19
	SD	1.6	0.41	0.19	0.21	0.5	1.26	0.874	1.136	0.101	0.000	0.022	108.8	0.5
4♀ 1000	57	55	15.0	7.0	27.3	79	5.5	0.88	4.51	0.00	0.00	0.11	1212	20
	58	56	14.8	6.9	26.4	81	9.3	3.35	5.95	0.00	0.00	0.00	760	CTD
	59 A	51	13.7	6.3	26.9	81	3.7	0.67	3.00	0.00	0.00	0.04	953	17
	60 A	56	15.2	7.1	27.1	79	10.2	1.63	8.36	0.10	0.00	0.10	714	19
	61	55	14.8	6.7	26.9	82	6.3	1.39	4.91	0.00	0.00	0.00	798	19
	Mean	55	14.7	6.8	26.9	80	7.0	1.58	5.35	0.02	0.00	0.05	887	19
	SD	2.1	0.58	0.32	0.33	1.3	2.70	1.059	1.990	0.045	0.000	0.053	202.5	1.2

SD Standard deviation
CTD Clotted Sample
P Slight polychromasia
A Slight anisocytosis

APPENDIX 4

Biochemistry - individual values

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	Glu-cose mg/dl	Protein g/dl Total	Alb	Glob	A/G	Urea Nitr mg/dl	Creat-inine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
1♂ Control	1	121	7.0	2.6	4.4	0.59	11	0.4	300	25	50	0.1	143	3.8	5.6	5.1	97	57
	2	113	6.4	2.6	3.8	0.68	11	0.5	555	27	58	0.1	144	3.5	5.6	4.7	99	65
	3	132	6.8	2.9	3.9	0.74	9	0.5	315	23	65	0.1	145	3.2	5.8	5.0	98	66
	4	113	6.6	2.6	4.0	0.65	10	0.5	325	25	56	0.1	144	3.5	5.7	4.7	99	66
	5	128	6.2	2.7	3.5	0.77	11	0.5	418	24	50	0.1	144	3.8	5.6	5.2	99	69
	Mean	121	6.6	2.7	3.9	0.69	10	0.5	383	25	56	0.1	144	3.6	5.7	4.9	98	65
	SD	8.6	0.32	0.13	0.33	0.072	0.9	0.04	106.9	1.5	6.3	0.00	0.7	0.25	0.09	0.23	0.9	4.5
2♂ 15	9	134	6.1	2.7	3.4	0.79	10	0.4	290	30	59	0.2	144	3.6	5.5	4.7	99	69
	10	125	6.6	2.7	3.9	0.69	10	0.5	437	27	53	0.1	146	3.6	5.6	4.8	102	52
	11	131	6.5	2.7	3.8	0.71	8	0.5	353	29	47	0.1	143	3.8	5.5	4.7	98	58
	12	153	6.9	2.7	4.2	0.64	9	0.3	397	35	53	0.1	142	4.7	5.6	4.9	99	70
	13	133	6.6	2.9	3.7	0.78	12	0.4	264	22	44	0.1	143	3.8	5.7	4.6	99	87
	Mean	135	6.5	2.7	3.8	0.72	10	0.4	348	29	51	0.1	144	3.9	5.6	4.7	99	67
	SD	10.5	0.29	0.09	0.29	0.063	1.5	0.08	72.1	4.7	5.8	0.04	1.5	0.46	0.08	0.11	1.5	13.4
3♂ 150	17	103	6.8	2.9	3.9	0.74	12	0.5	432	38	55	0.1	144	3.5	5.7	4.9	98	79
	18	114	6.7	2.8	3.9	0.72	10	0.5	300	27	51	0.1	146	3.4	5.6	4.8	100	62
	19	117	6.6	2.9	3.7	0.78	10	0.5	370	23	55	0.1	146	3.5	5.5	4.6	100	85
	20	100	6.3	2.8	3.5	0.80	9	0.5	316	29	53	0.1	147	3.3	5.6	5.2	100	65
	21	124	6.5	2.9	3.6	0.81	9	0.5	292	25	50	0.1	146	4.2	5.6	4.7	99	53
	Mean	112	6.6	2.9	3.7	0.77	10	0.5	342	28	53	0.1	146	3.6	5.6	4.8	99	69
	SD	10.0	0.19	0.05	0.18	0.039	1.2	0.00	58.8	5.8	2.3	0.00	1.1	0.36	0.07	0.23	0.9	13.0
4♂ 1000	25	97	6.5	3.1	3.4	0.91	14	0.6	419	31	57	0.1	145	3.9	5.7	5.1	100	52
	26	80	6.9	3.2	3.7	0.86	10	0.6	368	23	49	0.1	146	3.3	5.3	5.1	99	46
	27	99	6.7	2.9	3.8	0.76	8	0.5	408	27	48	0.1	145	3.6	5.6	4.8	100	79
	28	84	6.4	2.8	3.6	0.78	10	0.7	300	22	52	0.1	150	3.7	5.5	5.2	102	57
	29	83	6.5	2.9	3.6	0.81	10	0.5	377	22	53	0.1	147	4.2	5.5	5.0	99	73
	Mean	89	6.6	3.0	3.6	0.82	10	0.6	374	25	52	0.1	147	3.7	5.5	5.0	100	61
	SD	8.7	0.20	0.16	0.15	0.061	2.2	0.08	46.6	3.9	3.6	0.00	2.1	0.34	0.15	0.15	1.2	14.0

SD Standard deviation

APPENDIX 4

(Biochemistry - continued)

Week 4 (21 September 1994)

Group/ dosage mg/kg/day	Animal no.	Glu-cose mg/dl	Protein g/dl Total	Alb	Glob	A/G	Urea Nitr mg/dl	Creat-inine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
1♀ Control	33	118	6.5	2.8	3.7	0.76	13	0.5	205	21	40	0.2	144	3.5	5.7	4.4	100	49
	34	124	6.3	2.9	3.4	0.85	11	0.5	160	21	62	0.1	144	3.3	5.6	4.3	101	65
	35	124	7.1	3.2	3.9	0.82	9	0.5	258	31	50	0.1	145	4.0	5.8	3.9	102	77
	36	135	6.6	3.1	3.5	0.89	11	0.5	199	30	42	0.1	145	3.6	5.7	4.2	101	81
	37	129	7.1	3.0	4.1	0.73	14	0.5	358	32	52	0.1	145	3.7	5.6	3.4	102	92
	Mean	126	6.7	3.0	3.7	0.81	12	0.5	236	27	49	0.1	145	3.6	5.7	4.0	101	73
	SD	6.4	0.36	0.16	0.29	0.065	1.9	0.00	76.6	5.5	8.8	0.04	0.5	0.26	0.08	0.40	0.8	16.4
2♀ 15	41	106	6.9	3.2	3.7	0.86	15	0.5	246	31	61	0.2	146	3.3	5.7	4.4	101	75
	42	104	6.6	2.8	3.8	0.74	10	0.4	161	26	55	0.1	145	3.6	5.7	4.3	102	63
	43	116	7.0	3.3	3.7	0.89	13	0.4	124	26	54	0.1	145	3.6	5.6	4.0	102	79
	44	119	6.8	2.9	3.9	0.74	12	0.5	186	19	47	0.1	143	3.6	5.5	4.4	99	71
	45	114	6.3	3.0	3.3	0.91	13	0.5	149	25	49	0.1	144	3.4	5.8	4.3	99	80
	Mean	112	6.7	3.0	3.7	0.83	13	0.5	173	25	53	0.1	145	3.5	5.7	4.3	101	74
	SD	6.5	0.28	0.21	0.23	0.082	1.8	0.05	46.4	4.3	5.5	0.04	1.1	0.14	0.11	0.16	1.5	6.9
3♀ 150	49	128	7.2	2.8	4.4	0.64	10	0.5	374	23	43	0.1	142	3.3	5.6	4.2	99	59
	50	122	6.5	2.9	3.6	0.81	13	0.5	202	19	50	0.1	142	3.8	5.6	4.2	100	76
	51	113	7.2	3.2	4.0	0.80	10	0.6	178	26	57	0.1	145	3.6	5.5	3.7	103	65
	52	105	7.1	3.2	3.9	0.82	12	0.5	184	23	52	0.1	144	3.2	5.7	3.6	99	68
	53	120	6.7	3.0	3.7	0.81	13	0.5	152	23	51	0.1	146	3.3	5.4	4.3	101	79
	Mean	118	6.9	3.0	3.9	0.78	12	0.5	218	23	51	0.1	144	3.4	5.6	4.0	100	69
	SD	8.8	0.32	0.18	0.31	0.076	1.5	0.04	89.0	2.5	5.0	0.00	1.8	0.25	0.11	0.32	1.7	8.1
4♀ 1000	57	92	7.2	3.1	4.1	0.76	20	0.8	236	24	70	0.2	145	3.1	5.7	4.2	101	79
	58	110	7.6	3.1	4.5	0.69	11	0.6	281	22	53	0.1	144	3.4	5.8	4.3	100	98
	59	94	7.3	3.2	4.1	0.78	15	0.6	225	22	49	0.1	145	3.3	5.5	4.2	101	101
	60	104	7.3	3.1	4.2	0.74	13	0.5	179	18	42	0.2	143	3.4	5.8	4.1	100	85
	61	112	6.6	3.2	3.4	0.94	13	0.4	237	18	48	0.1	145	3.7	5.7	4.8	99	73
	Mean	102	7.2	3.1	4.1	0.78	14	0.6	232	21	52	0.1	144	3.4	5.7	4.3	100	87
	SD	9.1	0.37	0.05	0.40	0.094	3.4	0.15	36.4	2.7	10.6	0.05	0.9	0.22	0.12	0.28	0.8	12.0

SD Standard deviation

APPENDIX B

Organ weights - individual values

Week 5

Group/ dosage mg/kg/day	Animal no.	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Seminal Vesicle g	Epididymides Left g	Epididymides Right g	Prostate g
1♂ Control	1	484	2.15	24.1	1.23	3.94	53.3	1.889	1.880	1.03	0.569	0.535	0.80
	2	373	2.14	18.5	0.68	2.84	52.1	1.726	1.761	1.12	0.560	0.509	0.85
	3	387	2.05	20.5	0.84	2.94	53.7	1.705	1.691	1.15	0.511	0.463	0.79
	4	457	2.24	24.7	1.08	3.54	78.8	1.758	1.814	0.95	0.489	0.481	0.73
	5	447	2.31	23.8	0.84	3.90	63.7	1.829	1.897	1.43	0.494	0.489	0.91
	Mean	430	2.18	22.3	0.93	3.43	60.3	1.781	1.809	1.14	0.525	0.495	0.81
	SD	47.3	0.099	2.69	0.221	0.520	11.33	0.0763	0.0852	0.184	0.0375	0.0276	0.066
2♂ 15	9	359	1.98	15.6	0.75	2.83	55.2	1.506	1.516	0.89	0.468	0.461	0.80
	10	416	2.15	21.7	1.04	3.37	53.9	1.729	1.824	0.87	0.495	0.479	0.95
	11	451	2.13	24.1	1.13	3.58	60.8	1.703	1.665	1.79	0.556	0.543	1.05
	12	443	2.09	21.7	0.78	3.45	60.8	1.819	1.789	0.86	0.464	0.513	0.87
	13	380	2.11	18.7	0.67	3.02	61.9	1.542	1.535	0.97	0.496	0.491	0.62
	Mean	410	2.09	20.4	0.87	3.25	58.5	1.660	1.666	1.08	0.496	0.497	0.86
	SD	39.5	0.066	3.28	0.200	0.315	3.68	0.1318	0.1412	0.403	0.0368	0.0317	0.164
3♂ 150	17	412	2.09	20.0	0.76	3.50	65.2	1.866	1.849	1.10	0.496	0.491	0.59
	18	467	2.08	24.0	1.11	3.39	51.4	1.775	1.728	1.45	0.526	0.498	0.81
	19	416	2.14	19.8	0.74	3.48	52.5	1.814	1.739	0.88	0.437	0.459	0.72
	20	392	1.98	15.0	0.82	3.63	62.8	1.719	1.706	1.11	0.451	0.464	1.05
	21	470	2.05	21.1	0.78	3.46	55.6	1.775	1.782	0.89	0.534	0.523	0.61
	Mean	431	2.07	20.0	0.84	3.49	57.5	1.790	1.761	1.08	0.489	0.487	0.75
	SD	35.0	0.059	3.25	0.153	0.090	6.19	0.0544	0.0565	0.231	0.0436	0.0262	0.185
4♂ 1000	25	394	1.96	25.7	0.61	3.29	56.5	1.648	1.633	0.77	0.526	0.469	0.66
	26	358	2.23	26.0	0.72	3.59	64.5	1.729	1.782	1.19	0.553	0.487	0.75
	27	452	2.16	30.6	0.81	4.04	70.9	1.785	1.782	0.63	0.497	0.503	0.73
	28	397	2.14	24.7	0.88	3.51	65.9	1.820	1.831	0.80	0.565	0.513	0.92
	29	490	2.19	32.1	1.19	4.39	63.1	1.821	1.764	1.04	0.537	0.508	1.14
	Mean	418	2.13	27.8	0.84	3.76	64.2	1.761	1.758	0.88	0.536	0.496	0.84
	SD	52.6	0.106	3.31	0.218	0.444	5.20	0.0732	0.0744	0.226	0.0262	0.0180	0.192

SD Standard deviation

APPENDIX B

Organ weights - individual values

Week 5

Group/ dosage mg/kg/day	Animal no.	Body wt g	Brain g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Control	33	254	1.96	11.6	0.58	2.16	83.5	136.8
	34	270	1.92	11.8	0.66	2.31	86.0	105.4
	35	274	1.99	12.3	0.51	2.51	64.8	89.9
	36	266	1.84	12.3	0.67	2.79	70.6	127.5
	37	265	1.95	11.4	0.63	2.11	67.2	113.2
	Mean	266	1.93	11.9	0.61	2.38	74.4	114.6
	SD	7.4	0.055	0.40	0.067	0.281	9.69	18.41
2♀ 15	41	260	2.02	11.3	0.72	2.04	68.0	85.5
	42	262	2.02	12.4	0.65	2.50	87.9	108.7
	43	273	2.06	12.0	0.65	1.98	72.2	108.7
	44	252	1.90	10.2	0.63	2.08	75.8	106.4
	45	268	1.78	12.0	0.72	2.47	70.8	136.8
	Mean	263	1.96	11.6	0.67	2.21	74.9	109.2
	SD	8.0	0.117	0.88	0.044	0.250	7.77	18.25
3♀ 150	49	270	1.90	10.5	0.64	2.30	77.6	111.3
	50	293	1.98	11.6	0.70	2.69	77.0	136.6
	51	261	1.80	13.4	0.62	2.26	80.6	101.1
	52	248	1.90	12.9	0.62	2.35	88.5	99.2
	53	282	2.01	13.2	0.71	2.53	81.4	119.7
	Mean	271	1.92	12.3	0.66	2.43	81.0	113.6
	SD	17.7	0.082	1.22	0.046	0.181	4.59	15.29
4♀ 1000	57	284	1.92	21.6	0.61	3.02	87.2	100.5
	58	243	1.99	16.5	0.81	2.53	76.0	68.4
	59	277	1.91	17.8	0.66	2.77	76.4	112.4
	60	273	1.99	16.9	0.66	2.34	67.7	95.5
	61	290	1.93	20.9	0.89	2.58	93.0	107.3
	Mean	274	1.95	18.7	0.73	2.65	80.1	96.8
	SD	18.4	0.037	2.37	0.117	0.262	10.01	17.14

SD Standard deviation

APPENDIX 6

RESULTS OF THE FUNCTIONAL OBSERVATIONAL BATTERY

The data for the observation battery has been handled in the following manner. The observational endpoints such as ease of handling, activity, etc., have been tabulated for frequency of occurrence for each group. The following data was subjected to statistical analysis: rearing and activity counts, grip strength, hind limb splay, bodyweight and temperature.

Observational endpoints

Among males, there were only slight differences in the observational endpoints during the course of the study. During Week 4, however, the following observations were made:

> There was a low incidence of males at 1000 mg/kg/day showing slight body tremor during the recording of temperature. There was also an observation that in the righting response, some males were leaving one of their hindfeet tucked under the body. This was observed among all groups but the highest incidence was recorded at 1000 mg/kg/day.

Among females, there were clear effects of treatment at 1000 mg/kg/day by Week 4 including:

> One animal showed body tremor while being held in the hand.
> The incidence of facial staining was increased and in conjunction with this the majority of animals at this dosage appeared "badly groomed".
> In the arena, palpebral closure was observed in 4/8 animals. Body tremor was also observed (4/8 animals) with two of the animals classed has having moderate tremor.

Grip strength

There were no significant differences in forelimb grip strength.

There was a slight but significant increase in the hindlimb grip strength of males at 1000 mg/kg/day during Week 2. There were no significant differences at Week 4. Among females at 1000 mg/kg/day, there was a significant increase in hindlimb grip strength during Week 4.

Hindlimb splay

Hindlimb splay was significantly increased. Among males at 1000 mg/kg/day, a significant increase was confined to Week 2. Among females at 1000 mg/kg/day, however, hindlimb splay was significantly increased during Weeks 2 and 4.

Rearing counts

Among males, the frequency of rearing in the arena was considered comparable among the groups. Among females, it was noted that counts among females at 1000 mg/kg/day were slightly lower than controls during Week 4. Differences failed to attain statistical significance.

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APPENDIX 6

(Continued)

Activity counts

Among males, activity counts were variable among the groups. There was no indication of an effect of treatment. Among females, it was noted that counts among females at 1000 mg/kg/day were slightly lower than controls during Week 4. Differences failed to attain statistical significance.

Temperature

There were no significant differences in temperature among the groups.

Bodyweight

Bodyweights were generally comparable among the groups.

Summary of effects

Exposure to isodecyl benzoate was characterized by behavioural changes at 1000 mg/kg/day: These changes were generally not observed at Week 2 but were observed at Week 4.

The incidence of facial staining was increased among females at Week 4.
Badly groomed appearance was observed among females at Week 4.
The incidence of tremor was increased among males and females at Week 4.
The incidence of palpebral closure in the arena was increased among females at Week 4.
Hindlimb grip strength was increased among males at Week 2 and among females at Week 4.
Hindlimb splay was increased among males during Week 2 and among females during Week 2 and 4.

There was also a suggestion at Week 4 of decreased activity and rearing counts for females treated at 1000 mg/kg/day.

Discussion and Conclusion

Exposure to isodecyl benzoate for 28 days resulted in behavioural changes which were confined to 1000 mg/kg/day. Females appeared to be more affected than males. The behavioural changes included altered appearance (facial staining and badly groomed appearance), the induction of tremor, increased hindlimb grip strength and increased hindlimb splay.

To some extent the changes observed can be considered non-specific: changes in appearance and the suggestion of effects on activity/rearing. The changes in grip strength and foot splay could be considered as suggesting on an effect on motor components. However, the change in grip strength is for *increased* strength, rather than a decrease. There were also no obvious effects on gait. There is some effect on the nervous system in view of the observation of tremor.

At lower dosages, there was no clear evidence of an effect of treatment.

VCL/206

APPENDIX 6

(Continued)

Group summary of observations - males

	Predose				Week 2				Week 4			
Group	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
OBSERVATIONS:												
HOME CAGE												
posture, sitting	7	8	8	7	6	8	7	7	7	7	8	8
REMOVAL FROM CAGE												
removing, easy	8	8	8	8	7	8	8	8	8	8	8	8
handling, easy	6	7	7	7	8	8	8	8	7	8	7	7
salivation	1				1	1		2	1	1		1
vocalising					1	1			1	1		1
IN THE ARENA												
grooming	1	4	2	5	4	3	4	2	1	1	2	3
tremors												
slight	1	1	2	2	2	2	3	4	2	4	3	3
moderate										1		
arousal, alert	6	7	6	7	6	7	6	5	2	3	5	
piloerection												
defecation	4	5	3	2	2	2	1	1	3	3	2	2
urine present	2	3	4	3	3	3	2	1	4	3	6	4
GAIT												
walking on toes:				1		3	1	1	2	1	1	2
swaying						1					1	
limbs splayed												
hunched							1					1
unable to assess	1	1			2	1	2	3	5	4		
MANIPULATIONS												
approach, a reaction	6	6	5	7	7	7	7	7	4	4	8	5
touch, a reaction	5	4	6	6	6	5	7	3	3	1	6	5
startle (present)	8	8	8	8	8	8	8	8	6	7	6	7
righting, immediate	5	7	7	8	7	6	6	8	5	7	8	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	7	8	8	7	7	7	7	7	7
ADDITIONAL COMMENTS												
facial staining	3	5	2	3	1	2			1	1		
body groomed												
palpebral closure in arena	1						1	2	3	3	2	3
slight body tremors during temperature		1				2	2	1		1	1	3
position of feet during righting									1	1	2	3

Numbers reflect the number of animals either showing the response or with the indicated score

APPENDIX 6

VCL 206

(Continued)

Group summary of observations - females

	Predose				Week 2				Week 4			
Group	1	2	3	4	1	2	3	4	1	2	3	4
No. of animals	8	8	8	8	8	8	8	8	8	8	8	8
OBSERVATIONS:												
HOME CAGE												
posture, sitting	7	5	6	7	8	5	8	8	8	6	6	8
REMOVAL FROM CAGE												
removing, easy	7	8	8	8	7	7	7	8	8	7	7	8
handling, easy	6	4	6	5	6	5	7	7	5	6	5	6
salivation		2								2		
vocalising	2	3	1	2	1	4	2	2	2	2	2	1
tremors in the hand												1
IN THE ARENA												
grooming	5	2	1	6	3	4	4	3	2	2	4	5
tremors												
slight	1			1	2	1	1	1		1		2
moderate												2
arousal, alert	6	8	8	8	6	8	7	6	6	7	5	6
piloerection								1				
defecation				1								
urine present	1	1		1	1	1					1	
GAIT												
walking on toes:	3	3	4	2	4	5	6	6	4	5	6	5
swaying										1		
limbs splayed							1					1
hunched	1	1			1			3				
unable to assess	1											1
MANIPULATIONS												
approach, a reaction	7	6	6	7	8	7	8	7	8	6	8	6
touch, a reaction	7	4	6	5	6	6	5	6	5	5	5	3
startle (present)	8	8	8	8	8	8	8	8	6	6	6	7
righting, immediate	5	7	7	8	5	8	7	6	6	6	7	7
tail pinch, a reaction	8	8	8	8	8	8	8	8	8	8	8	8
pupil reflex (present)	8	8	8	8	7	8	8	8	8	7	7	8
ADDITIONAL COMMENTS												
facial staining	1	2	2	3	2	2	1	3		2	1	4
badly groomed												6
palpebral closure in arena	1							1				4
slight body tremors during temperature	1				2	1		1		1		1
position of feet during righting												

Numbers reflect the number of animals either showing the response or with the indicated score

APPENDIX 6

(Continued)

Group mean activity counts

Predose

Group	Mean activity counts	
	Males	Females
1	11	13
2	14	15
3	13	16
4	10	13

Week 2

Group	Mean activity counts	
	Males	Females
1	7	13
2	11	17
3	11	16
4	7	13

Week 4

Group	Mean activity counts	
	Males	Females
1	6	14
2	5	17
3	10	16
4	6	8

no statistical significance ($P > 0.05$)

APPENDIX 6

(Continued)

Group mean rearing counts

Predose

Group	Mean rearing counts	
	Males	Females
1	10	12
2	13	14
3	12	14
4	12	13

Week 2

Group	Mean rearing counts	
	Males	Females
1	8	13
2	10	17
3	11	16
4	8	12

Week 4

Group	Mean rearing counts	
	Males	Females
1	5	13
2	5	15
3	8	14
4	8	8

no statistical significance ($p > 0.05$)

APPENDIX 6

(Continued)

Group mean bodyweights

Predose

Group	Mean bodyweights (g)	
	Males	Females
1	241	188
2	239	192
3	241	194
4	240	191

Week 2

Group	Mean bodyweights (g)	
	Males	Females
1	346	235
2	344	240
3	352	244
4	342	244

Week 4

Group	Mean bodyweights (g)	
	Males	Females
1	417	267
2	405	267
3	429	271
4	402	271

no statistical significance ($P > 0.05$)

APPENDIX 6

(Continued)

Group mean forelimb grip strength

Predose

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	0.64	0.64
2	0.58	0.68
3	0.62	0.64
4	0.69	0.69

Week 2

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	0.95	0.87
2	0.90	0.94
3	0.94	0.86
4	1.00	0.90

Week 4

Group	Mean forelimb grip strength (kg)	
	Males	Females
1	1.05	1.00
2	1.11	1.00
3	1.15	0.97
4	1.16	1.02

no statistical significance ($P > 0.05$)

APPENDIX 6

(Continued)

Group mean hindlimb grip strength

Predose

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.60	0.67
2	0.55	0.68
3	0.56	0.70
4	0.64	0.74

Week 2

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.74	0.71
2	0.73	0.80
3	0.79	0.81
4	0.87+	0.85

Week 4

Group	Mean hindlimb grip strength (kg)	
	Males	Females
1	0.94	0.84
2	0.84	0.89
3	0.98	0.88
4	1.06	1.03+

+ $P < 0.05$

APPENDIX 6

(Continued)

Group mean splay values

Predose

Group	Mean splay values (cm)	
	Males	Females
1	9.4	7.8
2	10.2	9.0
3	8.3	8.8
4	9.3	9.0

Week 2

Group	Mean splay values (cm)	
	Males	Females
1	9.0	8.0
2	10.7	9.7
3	10.8	8.8
4	11.8+	10.6++

Week 4

Group	Mean splay values (cm)	
	Males	Females
1	9.4	8.4
2	10.3	9.3
3	11.0	9.1
4	10.4	11.1++

+ $P < 0.05$
++ $P < 0.01$

APPENDIX 6

(Continued)

Group mean temperature

Predose

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.2
2	37.9	38.3
3	37.9	38.4
4	37.9	38.1

Week 2

Group	Mean temperature (°C)	
	Males	Females
1	37.6	38.0
2	37.8	38.5
3	37.7	38.0
4	37.9	38.2

Week 4

Group	Mean temperature (°C)	
	Males	Females
1	37.8	38.5
2	38.2	38.4
3	37.8	38.3
4	37.9	37.9

no statistical significance ($P > 0.05$)

Animals showing occasional palpebral closure in the arena:

Week	Group	Animal no
Predose ♂		
	Group 1	6
	Group 2	no animals affected
	Group 3	no animals affected
	Group 4	no animals affected
Week 2♂	Group 1	no animals affected
	Group 2	no animals affected
	Group 3	24
	Group 4	31, 32
Week 4 ♂	Group 1	4, 5, 7
	Group 2	14, 15, 16
	Group 3	19, 24
	Group 4	28, 31, 32

Week	Group	Animal no
Predose ♀	Group 1	37
	Group 2	no animals affected
	Group 3	no animals affected
	Group 4	no animals affected
Week 2 ♀	Group 1	no animals affected
	Group 2	no animals affected
	Group 3	no animals affected
	Group 4	64
Week 4 ♀	Group 1	no animals affected
	Group 2	no animals affected
	Group 3	no animals affected
	Group 4	57, 58, 60, 64

KEY TO THE FUNCTIONAL OBSERVATIONAL BATTERY

Posture

- S Sitting/standing in cage
- R Rearing in cage
- Cl Climbing up cage

Ease of removal from cage

- 2 easy (little resistance)
- 3 slightly awkward

Ease of handling

- 2 easy (little resistance)
- 3 slightly awkward

Arousal

2,3,4,5 increasing levels of arousal with 4 being alert

Gait

- T Walking on toes
- A Swaying/lurching gait
- H Hindlimbs splayed
- HU Hunched

the numbers associated with gait indicate the degree of effect
1,2,3 increasing degree of effect

- U Unable to assess gait - insufficient movement to assess gait
- N Normal gait

Approach

- 1 No reaction
- 2 Sniffs
- 3 Approaches and sniffs
- 4 Freezes
- O Other

Touch

- 1 No reaction
- 2 Turns
- 3 Walks away
- 4 Freezes
- O Other

Startle

1	No reaction
2	Ear twitch
3	Noticeable flinch
4	Noticeable jump

Tail pinch

2	turns
	2 turns immediately
	3 violent turn
3	walks away
5	jumps forward

Righting reflex

1	immediate reaction
2	reaction slow

Tremors

N	No tremor observed
B	Body
H	Head
T	Tail

the numbers associated with tremors indicate the degree of effect
1,2,3 increasing degree of effect

Salivation, vocalising, grooming, pilo-erection

N	not observed
Y	sign observed

the associated numbers indicate the degree of response
1,2,3 increasing degree of effect

Pupil reflex

Y	reflex observed both pupils
L,R	Left or right pupil responded only
N	no reflex both pupils

Urine

S	Small amount observed
M	Moderate amount
L	Large amount

VCL/206 Predose

	Group 1 males							
Animal no	1	2	3	4	5	6	7	8
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	R	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	3	2	2	2	2	2	3
Salivation	N	N	N	N	N	Y1	N	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	D1	N	N	N	N	N	N	N
Grooming	N	N	Y	N	N	N	N	N
Activity count	3	8	16	8	1	7	19	23
Arousal	3	4	4	4	3	4	4	4
Rearing count	3	7	19	8	1	6	19	21
Bolus count	2	0	0	0	2	5	0	1
Urine present	M	M	N	N	N	N	N	N
Gait	N	N	N	N	U	N	N	N
MANIPULATIONS								
Approach	0	1	1	2	3	3	3	3
Touch	1	1	4	4	4	2	1	2
Startle	4	3	3	4	3	3	3	2
Righting reflex	2	1	1	1	1	2	2	1
Tail pinch	3	3	3	3	2	2	3	3
turns					2	2		
vocalises	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1
Pupil reflex	B	B	B	B	B	D	B	B
Temperature (C)	38.4	37.7	37.7	38.0	37.4	38.0	37.0	37.9
Bodyweight (g)	236	237	235	250	240	282	241	256
GRIP STRENGTH (kg)#								
Forelimb	0.80	0.90	0.57	0.54	0.61	0.55	0.63	0.62
Hindlimb	0.07	0.61	0.63	0.48	0.65	0.57	0.57	0.77
FOOTSPLAY (cm) #	9.1	10.1	8.0	11.0	10.9	8.1	6.6	11.5

Values represent the mean of two trials

YCL/206 Predose

	Group 2 males							
Animal no	9	10	11	12	13	14	15	16
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	B1
Grooming	Y	N	N	N	Y	N	Y	Y
Activity count	18	20	1	20	11	17	11	15
Arousal	4	4	3	4	4	4	4	4
Rearing count	23	13	0	16	13	11	15	10
Bolus count	2	3	4	3	0	0	0	1
Urine present	M	S	S	N	N	N	N	N
Gait	N	N	U	N	N	N	N	N
MANIPULATIONS								
Approach	3	3	1	3	3	2	3	1
Touch	4	3	4	2	1	1	1	1
Startle	4	3	3	3	3	2	3	3
Righting reflex	1	1	1	1	2	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises		Y2	Y2	Y2		Y2	Y1	
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (C)	38.2	38.1	38.1	37.6	37.6	38.1	38.4	37.2
Bodyweight (g)	222	239	241	243	233	240	231	259
GRIP STRENGTH (kg)#								
Forelimb	0.50	0.60	0.52	0.67	0.47	0.61	0.57	0.75
Hindlimb	0.50	0.69	0.66	0.58	0.38	0.56	0.49	0.58
FOOTSPLAY (cm) #	12.3	7.7	10.3	8.8	10.8	10.8	10.7	10.7

Values represent the mean of two trials

VCL/208 Predose

	Group 3 males							
Animal no	17	18	19	20	21	22	23	24
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	3
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	R1	B1	N	N	N	N
Grooming	N	N	N	Y	N	Y	N	N
Activity count	16	17	10	18	5	15	18	6
Arousal	4	4	4	4	4	5	4	3
Rearing count	18	18	10	15	9	10	18	3
Bolus count	2	4	5	0	0	0	0	0
Urine present	M	N	M	N	N	S	N	M
Gait	N	N	N	N	N	N	N	N
MANIPULATIONS								
Approach	3	2	3	1	3	1	3	1
Touch	2	4	5	1	4	3	2	1
Startle	4	3	3	3	3	4	3	3
Highting reflex	1	1	1	2	1	1	1	1
Tail pinch	3	3	5	5	3	3	3	3
turns								
vocalises	Y1	Y1	Y1	Y2	Y1		Y1	Y2
Pupil reflex	D	B	B	B	B	B	B	B
Temperature (C)	38.5	37.6	38.1	38.1	37.6	37.7	38.0	37.5
Bodyweight (g)	240	254	236	229	244	234	234	255
GRIP STRENGTH (kg)#								
Forelimb	0.59	0.76	0.80	0.45	0.37	0.69	0.45	0.90
Hindlimb	0.45	0.66	0.61	0.57	0.41	0.74	0.48	0.53
FOOTSPLAY (cm) #	8.0	8.5	9.2	8.8	5.5	9.9	6.1	11.4

Values represent the mean of two trials

VCL/206 Predose

	Group 4 males							
Animal no	25	26	27	28	29	30	31	32
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	R
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	3
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	N	B1	N	B1	N	N
Grooming	N	N	Y	Y	Y	N	Y	Y
Activity count	19	11	11	5	9	6	12	10
Arousal	4	4	4	3	4	4	4	4
Rearing count	19	15	14	5	10	7	14	11
Bolus count	0	4	0	3	0	0	0	0
Urine present	N	S	N	N	N	S	S	S
Gait	N	N	T1	N	N	N	N	N
MANIPULATIONS								
Approach	1	2	3	3	2	3	3	3
Touch	4	1	4	4	2	4	1	4
Startle	2	4	3	2	4	3	3	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
Turns								
vocalises		Y1	Y2		Y1	Y1	Y1	Y1
Pupil reflex	B	B	R	R	N	B	B	B
Temperature (°C)	38.4	38.1	37.8	38.1	37.7	37.5	37.7	38.2
Bodyweight (g)	244	231	243	239	247	237	239	241
GRIP STRENGTH (kg)#								
Forelimb	0.81	0.65	0.65	0.73	0.57	0.87	0.56	0.65
Hindlimb	0.69	0.80	0.64	0.73	0.49	0.73	0.54	0.54
FOOTSPLAY (cm) #	11.7	10.1	7.4	9.1	8.8	8.1	8.7	12.9

Values represent the mean of two trials

	Group 1 females							
Animal no	33	34	35	36	37	38	39	40
OBSERVATIONS								
IN THE CAGE								
Posture	S	CL	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	3	2	2
Handling	2	3	2	2	3	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	Y1	Y1	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	B1	N
Grooming	Y	Y	Y	N	Y	Y	N	N
Activity count	13	18	15	23	10	17	3	2
Arousal	5	4	4	4	4	4	4	3
Rearing count	3	18	18	19	6	22	5	1
Bolus count	0	0	0	0	0	0	0	0
Urine present	S	N	N	N	N	N	N	N
Gait	N	T1HU1	N	T1	N	T1	N	U
MANIPULATIONS								
Approach	2	3	3	2	1	2	2	3
Touch	4	2	4	2	1	4	3	4
Startle	4	4	3	3	3	4	3	3
Righting reflex	2	1	2	2	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y1		Y2	Y1	Y2	Y2		Y1
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (C)	38.7	38.0	38.2	38.3	38.0	38.3	37.9	38.5
Bodyweight (g)	179	193	191	192	185	188	180	193
GRIP STRENGTH (kg)#								
Forelimb	0.55	0.74	0.58	0.59	0.65	0.63	0.66	0.82
Hindlimb	0.64	0.85	0.59	0.60	0.71	0.71	0.64	0.55
FOOTSPLAY (cm) #	6.2	9.3	9.2	7.2	6.9	7.4	8.9	9.7

Values represent the mean of two trials

VCL/208 Predose

	Group 2 females							
Animal no	41	42	43	44	45	46	47	48
OBSERVATIONS								
IN THE CAGE								
Posture	S	R	S	S	S	R	CL	R
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	3	3	2	2	2	3	2	3
Salivation	N	Y1	N	N	N	N	N	N
Vocalising	N	N	Y1	N	N	Y1	Y1	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	N
Grooming	N	N	Y	N	Y	N	N	N
Activity count	17	10	20	14	14	17	13	13
Arousal	4	4	4	4	4	4	4	4
Rearing count	16	12	16	11	12	18	16	12
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	S	N	N
Gait	N	N	T1	N	N	T1	N	T1HU1
MANIPULATIONS								
Approach	3	2	1	1	3	2	0	2
Touch	4	1	1	1	1	3	0	2
Startle	3	3	4	3	3	3	3	3
Righting reflex	1	1	1	1	1	1	2	1
Tail pinch	3	3	3	3	3	3	0	3
turns								
vocalises	Y1	Y1	Y2	Y1	Y2	Y1		Y2
Pupil reflex	B	B	B	R	B	B	B	B
Temperature (C)	38.2	38.4	38.2	38.3	38.1	37.8	38.1	38.1
Bodyweight (g)	185	169	199	187	193	205	176	205
GRIP STRENGTH (kg)#								
Forelimb	0.62	0.72	0.76	0.58	0.56	0.75	0.60	0.68
Hindlimb	0.72	0.73	0.80	0.75	0.55	0.69	0.70	0.49
FOOTSPLAY (cm) #	7.7	10.5	10.1	8.3	7.8	7.3	8.2	11.4

Values represent the mean of two trials

VCL/206 Pretrose

	Group 3 females							
Animal no	49	50	51	52	53	54	55	56
OBSERVATIONS								
IN THE CAGE								
Posture	S	R	S	S	S	R	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	3	2	3	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	Y1	N	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	N
Grooming	N	N	N	N	Y	N	N	N
Activity count	19	13	14	14	22	13	15	18
Arousal	4	4	4	4	4	4	4	4
Rearing count	16	14	15	15	16	15	14	10
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	N	N	N
Gait	N	T1	T1	I1	N	N	N	T1
MANIPULATIONS								
Approach	2	3	3	1	2	1	2	3
Touch	1	4	4	3	1	3	4	3
Startle	3	3	4	3	3	3	3	2
Righting reflex	1	2	1	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y1	Y1	Y2	Y2	Y1		Y2	Y1
Pupil reflex	B	B	B	D	B	B	B	B
Temperature (C)	38.1	38.3	38.3	38.7	37.6	38.9	38.7	38.3
Bodyweight (g)	192	206	198	182	191	199	187	199
GRIP STRENGTH (kg)#								
Forelimb	0.73	0.53	0.64	0.57	0.43	0.74	0.75	0.71
Hindlimb	0.55	0.50	0.66	0.82	0.71	0.72	0.81	0.73
FOOTSPLAY (cm) #	11.5	8.5	8.8	8.4	7.3	7.4	11.5	7.9

Values represent the mean of two trials

	Group 4 females							
Animal no	57	58	59	60	61	62	63	64
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	R	S	R
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	3	2	2	2	3	3	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	Y1	Y2	N	N	N	N
IN THE ARENA								
Tremors	N	B1	N	N	N	N	N	N
Grooming	N	N	Y	Y	Y	Y	Y	Y
Activity count	18	9	13	22	15	11	4	14
Arousal	4	4	4	4	4	4	4	4
Rearing count	13	6	13	24	17	10	7	14
Bolus count	0	0	0	1	0	0	0	0
Urine present	N	N	N	S	N	N	N	N
Gait	N	T1	T1	N	N	N	N	N
MANIPULATIONS								
Approach	2	5	1	3	3	2	3	2
Touch	1	4	4	4	1	1	4	3
Startle	3	3	4	3	3	4	3	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	3	3	2	3	3	3	3
turns								
vocalises		Y2	Y1	Y2	Y1	Y2	Y2	
Pupil reflex	B	R	B	B	B	B	B	B
Temperature (C)	38.4	38.3	38.5	37.2	38.0	38.6	37.1	38.3
Bodyweight (g)	199	182	193	191	197	187	183	197
GRIP STRENGTH (kg)#								
Forelimb	0.69	0.70	0.72	0.73	0.67	0.72	0.74	0.61
Hindlimb	0.65	0.83	0.83	0.75	0.71	0.78	0.88	0.68
FOOTSPLAY (cm) #	11.1	9.2	11.3	7.3	8.9	7.6	8.8	9.8

Values represent the mean of two trials

VCL/206 Week 2

	Group 1 males							
Animal no	1	2	3	4	5	6	7	8
OBSERVATIONS								
IN THE CAGE								
Posture	R	S	S	S	S	S	R	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Salivation	N	N	N	N	N	N	N	Y1
Vocalising	N	N	N	N	Y2	N	N	N
IN THE ARENA								
Tremors	N	B1	N	B1	N	N	N	N
Grooming	Y	Y	Y	N	N	Y	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	6	9	12	5	3	9	2	9
Arousal	4	4	4	4	3	4	3	4
Rearing count	9	10	15	8	1	7	7	9
Bolus count	0	0	0	0	4	0	0	3
Urine present	N	M	N	N	S	N	N	S
Gait	N	N	N	N	U	N	U	N
MANIPULATIONS								
Approach	1	3	3	3	3	3	3	3
Touch	1	4	4	4	4	4	1	4
Startle	3	3	4	3	3	3	3	3
Righting reflex	2	1	1	1	1	1	1	1
Tail pinch	3	3	3	3	2	3	3	3
turns					3			
vocalises	Y2	Y2	Y2	Y1	Y3	Y1	Y2	Y2
Pupil reflex	B	B	B	B	D	B	B	B
Temperature (C)	38.0	37.8	37.5	37.7	37.2	37.2	37.1	38.2
Bodyweight (g)	372	320	342	365	352	320	336	362
GRIP STRENGTH (kg)#								
Forelimb	1.07	1.25	0.56	1.00	0.84	0.84	1.12	0.92
Hindlimb	0.71	0.70	0.71	0.58	0.69	0.66	0.74	0.93
FOOTSPLAY (cm) #	8.8	10.1	7.3	10.7	8.3	9.8	7.3	10.4

Values represent the mean of two trials

VCL/206 Week 2

	Group 2 males							
Animal no	9	10	11	12	13	14	15	16
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Salivation	N	Y1	N	N	N	N	N	N
Vocalising	N	N	Y1	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	H1B1T1	N	N	N	N	B1
Grooming	Y	Y	N	N	N	N	Y	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	21	19	1	18	4	10	5	6
Arousal	4	4	2	4	4	4	4	4
Rearing count	21	16	0	9	7	13	6	3
Bolus count	1	0	7	0	0	0	0	0
Urine present	M	S	M	N	N	N	N	N
Gait	T2	A1T1	U	T1	N	N	N	N
MANIPULATIONS								
Approach	5	3	1	3	3	3	3	3
Touch	4	4	4	4	1	3	1	1
Startle	3	4	3	3	2	3	2	3
Righting reflex	2	1	1	1	1	2	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y2	Y2	Y2	Y2	Y1	Y1	Y2	Y2
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (C)	37.8	38.5	38.0	37.3	36.9	37.9	38.0	38.0
Bodyweight (g)	304	349	358	361	331	337	347	352
GRIP STRENGTH (kg)#								
Forelimb	0.90	1.08	0.64	0.94	0.72	0.97	1.11	1.02
Hindlimb	0.69	0.84	0.77	0.61	0.59	0.69	0.79	0.90
FOOTSPLAY (cm) #	10.5	8.4	10.8	8.9	11.3	13.8	12.7	8.5

Values represent the mean of two trials

VCL/200 Week 2

	Group 3 males							
Animal no	17	18	19	20	21	22	23	24
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	R	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	B1	N	B1H1	N	N	N	B1
Grooming	Y	Y	Y	N	N	Y	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	18	6	12	1	17	15	14	1
Arousal	4	4	4	2	4	4	4	2
Rearing count	19	5	15	2	15	14	18	1
Bolus count	0	0	0	0	0	0	0	1
Urine present	N	N	N	N	N	M	S	M
Gait	N	N	HU1	U	T2	N	N	U
MANIPULATIONS								
Approach	3	2	3	3	3	2	3	1
Touch	3	1	3	2	4	4	3	4
Startle	3	3	3	3	3	3	3	4
Righting reflex	1	1	1	2	2	1	1	1
Tail pinch	3	3	3	5	3	3	3	3
turns								
vocalises	Y2	Y1	Y2		Y1	Y2	Y1	Y1
Pupil reflex	B	N	B	B	B	B	B	B
Temperature (C)	38.6	37.1	37.6	37.6	38.6	37.8	37.0	37.3
Bodyweight (g)	347	376	348	321	375	334	364	354
GRIP STRENGTH (kg)#								
Forelimb	1.10	1.09	1.08	0.80	0.59	0.92	0.90	1.29
Hindlimb	0.57	0.90	0.79	0.75	0.85	0.87	0.77	0.85
FOOTSPLAY (cm) #	11.6	10.3	10.8	7.1	10.1	13.5	9.2	13.6

Values represent the mean of two trials

VCI /206 Week 2

	Group 4 males							
Animal no	25	26	27	28	29	30	31	32
OBSERVATIONS								
IN THE CAGE								
Posture	R	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Salivation	N	N	Y1	N	N	N	Y1	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	B1	B1	N	N	B1	B1
Grooming	N	Y	N	N	Y	N	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	13	10	6	1	8	7	3	1
Arousal	4	4	4	8	4	4	3	2
Rearing count	13	14	6	1	11	12	3	1
Bolus count	0	0	0	8	0	0	0	0
Urine present	N	N	N	N	N	N	6	N
Gait	T1	N	N	U	N	N	U	U
MANIPULATIONS								
Approach	3	2	3	3	3	2	3	3
Touch	4	4	1	0	1	1	1	1
Startle	3	3	3	3	3	3	3	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y1	Y1	Y2			Y2	Y2	Y2
Pupil reflex	B	B	B	B	B	B	B	N
Temperature (C)	38.2	38.0	37.7	38.5	37.0	37.8	37.5	38.4
Bodyweight (g)	339	319	369	330	365	345	341	328
GRIP STRENGTH (kg)#								
Forelimb	1.06	0.98	0.98	1.15	0.67	1.14	0.92	1.10
Hindlimb	0.67	0.94	0.86	0.84	0.70	1.17	0.87	0.78
FOOTSPLAY (cm) #	11.7	13.4	10.3	8.7	14.0	13.7	8.2	14.3

Values represent the mean of two trials

VCL/206 Week 2

	Group 1 females							
Animal no	33	34	35	36	37	38	39	40
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	3	2	2	2
Handling	2	2	3	2	2	2	3	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	N	Y1	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	B1	B1
Grooming	Y	N	Y	N	N	Y	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	10	21	18	14	1	17	10	12
Arousal	4	4	4	4	3	4	5	4
Rearing count	12	11	16	17	1	22	13	10
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	M	N	N	N
Gait	N	T1	T1	T1HU1	U	T1	N	N
MANIPULATIONS								
Approach	3	3	3	3	3	2	3	3
Touch	2	3	1	2	3	1	3	3
Startle	3	3	3	3	3	3	3	3
Righting reflex	2	1	1	2	1	1	2	1
Tail pinch	3	3	3	0	3	3	3	3
turns						1		
vocalises	Y1	Y1	Y2	Y2		Y2		Y2
Pupil reflex	B	B	B	B	R	B	B	B
Temperature (C)	38.7	37.1	38.4	38.8	38.7	37.6	37.9	37.0
Bodyweight (g)	224	241	239	246	234	237	228	233
GRIP STRENGTH (kg)#								
Forelimb	0.79	0.91	1.02	0.83	0.85	0.76	0.81	1.02
Hindlimb	0.70	0.60	0.61	0.55	0.71	0.71	0.77	0.75
FOOTSPLAY (cm) #	8.1	7.8	9.7	8.0	9.9	8.2	5.6	8.8

Values represent the mean of two trials

VCL/206 Week 2

	Group 2 females							
Animal no	41	42	43	44	45	46	47	48
OBSERVATIONS								
IN THE CAGE								
Posture	R	OL	S	OL	S	S	S	S
IN THE HAND								
Removing	2	3	2	2	2	2	2	2
Handling	2	3	3	3	2	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	Y1	Y1	Y1	N	Y1	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	B1
Grooming	Y	Y	Y	N	Y	N	N	Y
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	27	18	17	12	20	12	19	9
Arousal	4	4	4	4	4	4	4	4
Rearing count	25	14	18	16	16	21	19	10
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	S	N	N	N	N	N
Gait	T2	N	T2	N	N	T2	T1	T1
MANIPULATIONS								
Approach	1	3	3	2	3	3	3	3
Touch	1	2	1	4	2	2	2	4
Startle	4	3	4	3	3	3	3	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	3	3	3	3	3	2	3
turns							2	
vocalises	Y1	Y1	Y2	Y2	Y2	Y2	Y2	Y3
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (C)	39.0	39.4	38.5	38.3	38.4	37.8	38.0	37.8
Bodyweight (g)	229	227	254	230	247	252	213	254
GRIP STRENGTH (kg)#								
Forelimb	0.85	1.19	1.12	1.02	0.66	0.95	0.67	1.05
Hindlimb	1.02	0.85	1.09	0.93	0.53	0.83	0.48	0.72
FOOTSPLAY (cm) #	9.5	11.7	10.8	9.6	7.7	8.9	7.6	11.9

Values represent the mean of two trials

VCL/206 Week 2

	Group 3 females							
Animal no	49	50	51	52	53	54	55	56
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	3	2	2	2
Handling	2	2	2	2	3	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	Y1	N	N	Y1	N	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	B1	N
Grooming	Y	Y	N	N	N	Y	N	Y
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	12	14	13	19	27	14	13	16
Arousal	4	4	4	4	5	4	4	4
Rearing count	12	11	20	16	23	11	17	19
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	N	N	N
Gait	T1	T1	N	T2	T2	T2H1	N	T1
MANIPULATIONS								
Approach	3	3	3	3	0	3	3	3
Touch	1	3	4	2	1	1	4	2
Startle	3	3	4	3	4	3	3	3
Righting reflex	1	2	1	1	1	1	1	1
Tail pinch	3	3	3	5	5	3	3	3
turns			1					
vocalises	Y2	Y2	Y2	Y2	Y1	Y2	Y2	Y1
Pupil reflex	B	B	D	B	B	B	B	B
Temperature (C)	37.0	37.3	30.2	38.2	39.2	37.9	37.1	37.6
Bodyweight (g)	239	264	235	225	248	260	228	252
GRIP STRENGTH (kg)#								
Forelimb	0.84	1.05	0.85	0.86	0.86	0.90	0.87	0.89
Hindlimb	0.60	1.01	0.66	1.08	0.70	0.95	0.77	0.74
FOOTSPLAY (cm) #	9.0	11.4	10.2	5.2	7.2	8.1	9.4	9.7

Values represent the mean of two trials

VCL/206 Week 2

	Group 4 females							
Animal no	57	58	59	60	61	62	63	64
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	3	2	2	2	2	2
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	Y2	Y1	N	N	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	B1
Grooming	Y	N	N	N	Y	N	Y	N
Pilo-erection	N	N	Y	N	N	N	N	N
Activity count	17	6	21	18	19	10	8	8
Arousal	4	3	4	4	4	4	4	3
Rearing count	7	5	22	16	19	10	13	4
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	N	N	N
Gait	T1HU1	N	T3HU1	T2	T1HU2	T1	T1	N
MANIPULATIONS								
Approach	1	3	3	3	3	3	3	3
Touch	4	1	2	3	3	4	1	4
Startle	3	3	3	3	3	2	3	3
Righting reflex	1	1	2	1	1	1	2	1
Tail pinch	3	3	0	3	3	3	3	2
turns								2
vocalises	Y1	Y2	Y1	Y2	Y2	Y2	Y2	Y2
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (°C)	37.5	38.2	39.1	38.0	39.2	38.6	37.6	37.7
Bodyweight (g)	258	227	241	249	254	249	228	244
GRIP STRENGTH (kg)#								
Forelimb	0.85	0.84	0.91	1.04	0.93	0.83	0.94	0.88
Hindlimb	0.77	0.94	0.94	0.71	0.88	0.76	0.94	0.88
FOOTSPLAY (cm) #	10.3	9.8	10.4	10.8	10.0	10.4	12.8	10.5

Values represent the mean of two trials

VCL/206 Week 4

	Group 1 males							
Animal no	1	2	3	4	5	6	7	8
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	R	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	3	2	2	2	2
Tremors	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	Y1	N	N
Vocalising	N	N	N	N	Y2	N	N	N
IN THE ARENA								
Tremors	N	S1	N	S1	N	N	N	N
Grooming	N	N	N	N	N	N	N	Y
Pilo erection	N	N	N	N	N	N	N	N
Activity count	1	2	13	1	1	6	1	25
Arousal	2	3	4	2	2	3	3	4
Rearing count	0	4	13	0	0	5	3	11
Bolus count	4	2	0	0	4	0	0	0
Urine present	N	S	S	N	S	S	N	N
Gait	U	U	11	U	U	N	U	T2
MANIPULATIONS								
Approach	3	0	2	5	1	1	1	1
Touch	1	0	1	0	1	1	1	4
Startle	4	3	3	2	3	3	3	3
Righting reflex	2	1	1	1	2	1	2	1
Tail pinch	3	3	3	3	2	3	3	3
turns					2		1	
vocalises		Y2	Y1	Y2	Y2	Y1	Y2	Y2
Pupil reflex	B	D	B	L	B	B	B	B
Temperature (C)	38.3	38.0	37.6	38.1	38.9	37.9	37.7	37.8
Bodyweight (g)	491	378	389	480	433	371	383	497
GRIP STRENGTH (kg)#								
Forelimb	1.14	1.18	0.84	1.21	0.78	1.05	1.09	1.13
Hindlimb	0.87	0.92	0.64	1.07	1.06	0.96	0.91	1.14
FOOTSPLAY (cm) #	9.7	10.3	9.5	10.9	8.0	9.2	6.4	12.4

Values represent the mean of two trials

VCL/206 Week 4

	Group 2 males							
Animal no	9	10	11	12	13	14	15	16
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	R	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	2	2	2	2	2
Tremors	N	N	N	N	N	N	N	N
Salivation	N	N	N	Y1	N	N	N	N
Vocalising	N	N	Y2	N	N	N	N	N
IN THE ARENA								
Tremors	N	B1	B1	N	N	B1	B1	B2
Grooming	N	N	N	N	N	N	Y	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	13	4	1	16	3	1	1	1
Arousal	4	3	2	4	4	3	3	2
Rearing count	7	3	0	15	10	1	2	0
Bolus count	3	4	6	0	0	0	0	0
Urine present	M	S	N	N	N	S	N	N
Gait	N	N	U	T2	N	U	U	U
MANIPULATIONS								
Approach	1	1	1	3	3	2	3	1
Touch	1	1	1	1	1	4	1	1
Startle	2	3	3	3	3	3	3	3
Righting reflex	1	1	1	1	1	1	1	2
Tail pinch	3	3	2	3	3	3	3	3
turns			2					
vocalises	Y2	Y2	Y2	Y2		Y2	Y1	Y2
Pupil reflex	B	B	B	B	B	R	R	R
Temperature (°C)	38.4	38.4	38.8	37.9	37.8	37.7	38.1	38.1
Bodyweight (g)	357	415	438	439	379	383	403	423
GRIP STRENGTH (kg)#								
Forelimb	0.99	1.29	0.96	1.37	1.11	1.13	1.19	0.89
Hindlimb	0.99	0.98	0.82	0.80	0.69	0.87	0.78	0.85
FOOTSPLAY (cm) #	11.9	11.2	8.9	8.5	11.2	11.0	10.0	9.6

Values represent the mean of two trials

VCL/206 Week 4

	Group 3 males							
Animal no	17	18	19	20	21	22	23	24
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	3	2	2	2	2	2	2
Tremors	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	N	N	N	N
IN THE ARENA								
Tremors	N	B1	B1	B1	N	N	N	N
Grooming	Y	N	Y	N	N	Y	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	18	2	5	5	17	16	16	1
Arousal	4	3	3	4	4	4	4	2
Rearing count	15	1	6	6	10	11	15	0
Bolus count	0	0	4	0	0	0	0	4
Urine present	S	N	S	S	N	M	S	S
Gait	N	U	N	N	T2A1	N	N	U
MANIPULATIONS								
Approach	3	2	3	3	3	3	3	5
Touch	3	1	3	3	3	1	2	2
Startle	2	3	3	3	3	3	2	3
Righting reflex	1	1	1	1	1	1	1	1
Tail pinch	3	2	3	5	2	3	3	3
turns		2			2			
vocalises	Y2	Y2	Y2	Y2	Y3	Y2	Y1	Y2
Pupil reflex	R	B	R	R	B	B	B	B
Temperature (C)	38.5	30.4	37.8	37.6	37.9	37.0	38.1	37.1
Bodyweight (g)	415	467	418	395	476	400	448	411
GRIP STRENGTH (kg)#								
Forelimb	1.24	1.41	1.40	0.98	0.91	0.78	1.13	1.39
Hindlimb	0.71	1.04	1.12	1.04	1.02	1.08	0.96	0.91
FOOTSPLAY (cm) #	12.5	12.6	9.7	8.5	13.8	11.2	9.1	11.3

Values represent the mean of two trials

VCL/206 Week 4

	Group 4 males							
Animal no	25	26	27	28	29	30	31	32
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	2	3	2	2	2	2
Tremors	N	N	N	N	N	N	N	N
Salivation	N	N	Y1	N	N	N	N	N
Vocalising	N	N	N	Y2	N	N	N	N
IN THE ARENA								
Tremors	N	N	B1	B2	N	N	B1	B1
Grooming	N	N	N	N	Y	N	Y	Y
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	12	7	9	1	10	4	2	6
Arousal	4	4	4	2	4	3	4	4
Rearing count	16	5	13	1	6	5	4	12
Bolus count	0	1	0	4	0	0	0	0
Urine present	S	S	N	N	S	N	S	N
Gait	T1	N	T1HU1	U	N	N	N	N
MANIPULATIONS								
Approach	3	2	1	2	3	1	2	1
Touch	0	4	1	0	2	1	4	1
Startle	3	3	3	3	3	2	3	3
Righting reflex	1	1	1	1	1	2	1	1
Tail pinch	3	3	3	3	2	3	3	3
turns					2			
vocalises	Y1	Y2	Y2	Y2		Y3	Y1	
Pupil reflex	B	B	B	B	B	R	B	R
Temperature (C)	39.1	38.5	38.3	38.0	36.8	37.7	36.8	37.7
Bodyweight (g)	388	354	443	391	482	392	392	374
GRIP STRENGTH (kg)#								
Forelimb	1.33	1.11	1.17	1.18	0.88	1.29	1.14	1.37
Hindlimb	1.20	1.23	1.10	1.22	0.92	1.10	0.85	0.85
FOOTSPLAY (cm) #	11.5	12.0	11.3	9.9	8.1	9.6	8.7	12.2

Values represent the mean of two trials

VCL/206 Week 4

	Group 1 females							
Animal no	33	34	35	36	37	38	39	40
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	3	2	2	3	2	3	2
Tremors	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	N	Y1	Y1	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	N
Grooming	Y	N	Y	N	N	N	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	7	20	19	12	1	25	19	10
Arousal	5	4	4	4	3	4	4	4
Rearing count	5	15	21	13	1	24	19	8
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	N	N	N
Gait	N	T1	T2	N	U	N	T1	T1
MANIPULATIONS								
Approach	2	3	0	3	3	3	3	3
Touch	4	3	1	3	1	0	0	1
Startle	4	3	3	2	3	3	3	3
Righting reflex	2	1	1	1	1	1	1	2
Tail pinch	3	3	3	3	5	2	3	3
turns						2		
vocalises	Y1		Y2	Y1	Y1	Y2	Y1	Y2
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (°C)	38.5	39.1	38.5	38.9	37.2	39.4	38.2	38.2
Bodyweight (g)	256	273	273	275	271	261	252	272
GRIP STRENGTH (kg)#								
Forelimb	0.94	1.14	1.10	0.89	1.06	0.90	0.77	1.17
Hindlimb	0.73	0.69	1.05	0.68	0.89	0.86	0.80	0.98
FOOTSPLAY (cm) #	7.6	11.1	8.3	8.9	7.7	7.6	7.1	9.5

Values represent the mean of two trials

VCL/206 Week 4

	Group 2 females							
Animal no	41	42	43	44	45	46	47	48
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	CL	S	S	R	S
IN THE HAND								
Removing	3	2	2	2	2	2	2	2
Handling	3	2	2	3	2	2	2	2
Tremors	N	N	N	N	N	N	N	N
Salivation	Y1	N	Y1	N	N	N	N	N
Vocalising	Y1	N	Y1	N	N	N	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	BI
Grooming	Y	N	N	N	Y	N	N	N
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	23	1	24	19	24	10	25	7
Arousal	4	2	4	4	4	4	4	4
Rearing count	19	0	27	19	10	12	17	4
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	N	N	N
Gait	T2A1	U	T2	T1	N	T1	T2	N
MANIPULATIONS								
Approach	2	1	2	1	3	3	5	2
Touch	4	1	4	1	2	2	4	1
Startle	4	3	3	3	3	3	2	3
Righting reflex	1	1	1	1	1	2	2	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y2	Y1	Y2	Y2	Y2	Y2	Y2	Y3
Pupil reflex	B	B	B	B	B	B	B	R
Temperature (C)	38.5	39.0	38.6	39.0	37.7	38.2	39.2	37.2
Bodyweight (g)	265	250	280	255	267	282	232	294
GRIP STRENGTH (kg)#								
Forelimb	0.64	1.15	1.15	1.05	0.95	1.15	0.92	1.07
Hindlimb	0.96	1.06	1.04	1.03	0.80	0.70	0.66	0.69
FOOTSPLAY (cm) #	8.1	10.3	9.7	10.2	8.5	8.0	8.4	10.7

Values represent the mean of two trials

VCL/206 Week 4

	Group 3 females							
Animal no	49	50	51	52	53	54	55	56
OBSERVATIONS								
IN THE CAGE								
Posture	S	R	S	CL	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	3	2	2	2
Handling	2	2	3	3	3	2	2	2
Tremors	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	N	Y2	Y1	N	N	N
IN THE ARENA								
Tremors	N	N	N	N	N	N	N	N
Grooming	Y	Y	Y	N	N	N	N	Y
Pilo-erection	N	N	N	N	N	N	N	N
Activity count	11	26	6	28	11	19	8	18
Arousal	4	4	3	4	3	4	3	4
Rearing count	10	90	8	26	4	20	6	16
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	S	N	N	N	N
Gait	T1	T1	N	T2	I1	T1	T1	N
MANIPULATIONS								
Approach	3	3	3	3	3	3	3	3
Touch	1	3	3	2	1	1	3	2
Startle	3	3	2	2	3	3	3	3
Righting reflex	1	2	1	1	1	1	1	1
Tail pinch	3	3	2	3	2	3	3	3
turns	1		2		3			
vocalises	Y2	Y2		Y1	Y2	Y1	Y2	Y1
Pupil reflex	L	B	B	B	D	B	B	B
Temperature (C)	39.2	38.8	38.0	39.2	38.0	38.0	37.9	37.8
Bodyweight (g)	270	291	263	243	277	290	251	284
GRIP STRENGTH (kg)#								
Forelimb	0.90	1.12	1.00	0.92	0.84	0.81	0.98	1.12
Hindlimb	0.75	0.90	1.02	0.73	1.05	0.98	0.90	0.74
FOOTSPLAY (cm) #	9.5	10.8	10.5	4.6	10.9	9.4	9.5	8.0

Values represent the mean of two trials

	Group 4 females							
Animal no	57	58	59	60	61	62	63	64
OBSERVATIONS								
IN THE CAGE								
Posture	S	S	S	S	S	S	S	S
IN THE HAND								
Removing	2	2	2	2	2	2	2	2
Handling	2	2	3	2	3	2	2	2
Tremors	B1	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Vocalising	N	N	Y1	N	N	N	N	N
IN THE ARENA								
Tremors	D2	D2	N	N	N	N	B1	B1
Grooming	Y	N	Y	Y	Y	N	N	Y
Pilo-erection	N	N	Y	N	N	N	N	N
Activity count	7	1	18	14	5	6	5	5
Arousal	4	2	4	4	5	4	4	4
Rearing count	4	1	13	17	14	5	2	5
Bolus count	0	0	0	0	0	0	0	0
Urine present	N	N	N	N	N	N	N	N
Gait	N	U	T3H1	T2	T1	N	T1	T1
MANIPULATIONS								
Approach	1	2	3	3	3	2	3	1
Touch	1	1	2	4	2	1	1	1
Startle	4	3	3	3	3	3	3	3
Righting reflex	1	1	1	2	1	1	1	1
Tail pinch	3	3	3	3	3	3	3	3
turns								
vocalises	Y2	Y2	Y2	Y2	Y1	Y1	Y2	Y2
Pupil reflex	B	B	B	B	B	B	B	B
Temperature (C)	38.2	37.9	37.7	37.1	38.7	39.0	37.4	37.4
Bodyweight (g)	268	247	271	259	287	276	258	270
GRIP STRENGTH (kg)#								
Forelimb	1.06	0.90	0.96	1.16	0.98	0.97	1.20	1.00
Hindlimb	1.07	0.94	1.12	1.05	1.15	0.82	0.96	1.11
FOOTSPLAY (cm) #	11.0	10.9	13.7	11.4	8.3	9.1	12.6	11.6

Values represent the mean of two trials