APPENDIX 1

Analysis of Enzyme preparation from *Aspergillus niger* (ARO-1) - certificate from the Sponsor

Gist-brocades
BIOTECHNOLOGY CONTRIBUTING TO FOOD, HEALTH AND THE ENVIRONMENT

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CABLES GISTBROCADES

CERTIFICATE OF ANALYSIS

name of the product	:	Enzyme preparation from *Aspergillus niger* ARO-1
batch no.	:	RER 710
GLP-archive no.	:	GLP-9708
status	:	ISO 9002
date of manufacture	:	July 1997
date of expiration	:	October 1999 (provisional)
active component(s)	:	pectinase, apiosidase, arabinofuranosidase, β-glucosidase, rhamnosidase
date of issue	:	22 February 1999

analysis type	method no.	result	specification
1 Pectinase activity	CQA 4 005 00	31,600 AVJP/g	record
2 β-D-glucosidase activity	CQA 4 046 00	1750 BDG/g	record
3 Apiosidase activity	R+D 4483 3/10/94	498 nK/g	record
4 Arabinofuranosidase activity	CQA 4 054 00	14,800 ARF/g	record
5 Rhamnosidase activity	CQA 4 106 00	84 RHU/g	record
6 Chlorogenase activity	CQA 4 135 00	14.8 µmol/h/g	record
7 Stability at 21°C during 48 hrs (50, 150 and 500 mg/ ml in water)	CQA 4 046 00	> 90%	record
8 Stability at 4°C during 15 days (50, 150 and 500 mg/ ml in water)	CQA 4 046 00	> 90%	record
9 Description	CQA 7 022 00	clear liquid	record
10 Colour	CQA 7 022 00	brown	record
11 Dry matter	60335	9.1% (w/w)	record
12 Ashes	60328	0.3% (w/w)	record
13 Total protein (N*6.25)	60055	5.1% (w/w)	record
14 Total carbohydrates	calculate	3.7% (w/w)	record
15 Total organic solids	SOP 869/E	8.8% (w/w)	record
16 Antifoam	W 0660.A	PEG: 15 mg/kg PPG: 46 mg/kg	record
17 Heavy metals (as Pb)	FCC IV	< 30 mg/kg	≤ 30 mg/kg

Study Manager

Release: yes / no

(b) (6)

date: 22-02-1999

GIST-BROCADES B.V.
TRADE REGISTER
DELFT NR 27704
ESTABLISHED AT DELFT

Gist-brocades
BIOTECHNOLOGY CONTRIBUTING TO FOOD, HEALTH AND THE ENVIRONMENT

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CABLES GISTBROCADES

CERTIFICATE OF ANALYSIS

name of the product	:	Enzyme preparation from *Aspergillus niger* ARO-1
batch no.	:	RER 710
GLP-archive no.	:	GLP-9708
status	:	ISO 9002
date of manufacture	:	July 1997
date of expiration	:	October 1999 (provisional)
active component(s)	:	pectinase, apiosidase, arabinofuranosidase, β-D-glucosidase, rhamnosidase
date of issue	:	22 February 1999

analysis type	method no.	result	specification
18 Lead	60401	< 5 mg/kg	≤ 5 mg/kg
19 Arsenic	61748	< 3 mg/kg	≤ 3 mg/kg
20 Cadmium	60988	< 0.5 mg/kg	≤ 0.5 mg/kg
21 Mercury	61748	< 0.5 mg/kg	≤ 0.5 mg/kg
22 Antimicrobial activity	69811	absent[1]	absent by test
23 Standard plate count	69814	< 5 CFU/g	< 10^6 CFU/g
24 Coliforms	69817	< 10 CFU/g	< 30 CFU/g
25 Salmonella	69825	absent/25 g	absent by test
26 Escherichia coli	69849	absent/25 g	absent by test
27 *Staphylococcus aureus*	69803	absent/g	absent by test
28 Aflatoxin B1	71 3360.00	absent by test	absent by test
29 T2 toxin	71 3361.00	absent by test	absent by test
30 Ochratoxin A	71 3362.00	absent by test	absent by test
31 Zearalenone	71 3363.00	absent by test	absent by test

Study Manager

remark: [1] one with ∅ = 18 mm

Release: yes / ~~no~~

(b) (6)

date: 22-02-1999

APPENDIX 2

Signs - individual observations

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 060

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY / KEYWORD / QUALIFIER (GROUP: 2M)	DAYS 1-8
10	7	2	COAT	
			HAIRLOSS	
			DORSAL BODY SURFACE	8
			SKIN	
			SCAB	
			LEFT UPPER DORSAL THORAX	3, 8

APPENDIX 2 - continued.

Signs - individual observations

Group : 1 2 3 4
Compound : Control ---- ARD-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

Schedule number: GSB 060

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 1F DAYS 1-8
23	7	2	BEHAVIOUR	
			IRRITABLE	3
			VOCALIZATION	3, 8

APPENDIX 2 - continued.

Signs - individual observations

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

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Schedule number: GSB 060

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER	GROUP: 3F DAYS 1-8
35	7	2	BEHAVIOUR VOCALIZATION	8

APPENDIX 2 - continued.

Signs - individual observations

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER	DEATH CODE	WK OF DEATH	CATEGORY KEYWORD QUALIFIER (GROUP: 4F)	DAYS 1-8
38	7	2	BEHAVIOUR VOCALIZATION	8

APPENDIX 3

Bodyweights - individual values (g)

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Schedule number: GSB 060

Group	:	1	2	3	4
Compound	:	Control	----	ARO-1	----
Dosage (mg/kg/day)	:	0	500	1500	5000

GROUP	ANIMAL	DAY 0	DAY 3	DAY 7
1M	1	163	187	227
	2	173	192	230
	3	185	202	245
	4	182	205	241
	5	163	180	214
2M	6	185	210	252
	7	189	214	259
	8	183	206	248
	9	183	205	249
	10	192	217	265
3M	11	177	199	232
	12	181	205	250
	13	168	188	221
	14	174	195	231
	15	182	203	233
4M	16	179	200	234
	17	197	225	263
	18	169	196	228
	19	159	181	212
	20	168	188	223

APPENDIX 3 - continued.

Bodyweights - individual values (g)

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Schedule number: GSB 060

Group		: 1	2	3	4
Compound		: Control	----	ARO-1	----
Dosage (mg/kg/day)		: 0	500	1500	5000

GROUP	ANIMAL	DAY 0	DAY 3	DAY 7
1F	21	152	161	183
	22	164	177	199
	23	150	158	176
	24	154	159	180
	25	171	183	208
2F	26	154	162	181
	27	159	171	188
	28	169	176	191
	29	159	166	183
	30	146	157	176
3F	31	167	182	200
	32	161	175	192
	33	168	177	194
	34	157	165	181
	35	172	188	203
4F	36	159	168	183
	37	141	147	160
	38	157	172	194
	39	154	163	179
	40	168	183	205

APPENDIX 4A

Absolute organ weights - individual values (g) for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	BRAIN	KIDNEYS	EPIDIDYMID	LIVER	SPLEEN	TESTES	THYMUS	THYROIDS+P
1M	1	222.0	0.034	1.75	2.02	0.225	13.1	0.673	2.52	0.596	0.011
	2	226.8	0.033	1.93	2.14	0.331	11.2	0.577	2.68	0.642	0.010
	3	240.8	0.042	1.88	2.01	0.265	14.3	0.775	3.55	0.547	0.012
	4	234.6	0.036	1.72	2.22	0.327	13.6	0.539	2.33	0.520	0.010
	5	212.2	0.030	1.88	1.89	0.216	10.5	0.559	1.96	0.382	0.009
2M	6	247.7	0.037	1.76	2.20	0.284	13.7	0.711	2.30	0.575	0.012
	7	255.3	0.035	1.84	2.21	0.303	15.2	0.785	2.32	0.535	0.014
	8	246.5	0.036	1.96	2.24	0.361	12.4	0.695	2.42	0.731	0.008
	9	243.6	0.030	1.90	2.18	0.321	12.2	0.596	2.44	0.701	0.009
	10	259.1	0.039	1.73	2.03	0.233	16.3	0.708	2.34	0.756	0.007
3M	11	227.1	0.032	1.87	1.97	0.269	12.2	0.676	2.57	0.696	0.012
	12	243.5	0.038	1.87	2.07	0.225	13.0	0.740	2.22	0.490	0.011
	13	220.6	0.035	1.72	1.80	0.204	10.7	0.492	0.90	0.621	0.009
	14	225.5	0.036	1.89	2.12	0.295	11.6	0.677	2.58	0.614	0.006
	15	231.5	0.043	1.86	2.08	0.311	11.5	0.716	2.29	0.516	0.012
4M	16	229.1	0.035	1.82	1.93	0.264	12.5	0.639	1.99	0.619	0.015
	17	262.3	0.039	1.92	2.13	0.268	12.7	0.678	2.45	0.579	0.011
	18	223.1	0.026	1.86	1.92	0.272	11.6	0.587	2.09	0.624	0.013
	19	208.4	0.036	1.85	1.87	0.290	11.3	0.622	2.60	0.557	0.007
	20	218.0	0.039	1.82	1.79	0.308	11.2	0.567	2.68	0.583	0.008

APPENDIX 4A - continued.

Absolute organ weights - individual values (g) for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	HEART	LUNGS & BR
1M	1	222.0	0.92	1.14
	2	226.8	1.10	1.26
	3	240.8	1.06	1.21
	4	234.6	1.00	1.26
	5	212.2	0.88	1.23
2M	6	247.7	1.14	1.43
	7	255.3	1.05	1.25
	8	246.5	0.99	1.41
	9	243.6	1.20	1.30
	10	259.1	1.07	1.32
3M	11	227.1	1.11	1.16
	12	243.5	1.08	1.34
	13	220.6	1.29	1.27
	14	225.5	0.99	1.46
	15	231.5	0.99	1.13
4M	16	229.1	1.05	1.53
	17	262.3	1.10	1.59
	18	223.1	1.08	1.41
	19	208.4	0.95	1.52
	20	218.0	0.95	1.51

APPENDIX 4A - continued.

Absolute organ weights - individual values (g) for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	BRAIN	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	THYROIDS+P	UTERUS + C
1F	21	178.3	0.038	1.76	1.60	8.4	0.061	0.453	0.475	0.011	0.71
	22	194.8	0.050	1.77	1.63	9.4	0.049	0.654	0.689	0.009	0.35
	23	175.3	0.031	1.78	1.66	8.8	0.065	0.543	0.547	0.012	0.32
	24	180.5	0.034	1.70	1.83	9.2	0.074	0.530	0.443	0.012	0.36
	25	209.5	0.060	1.87	1.74	10.2	0.074	0.854	0.720	0.007	0.34
2F	26	178.6	0.043	1.82	1.55	9.2	0.049	0.535	0.794	0.011	0.25
	27	185.7	0.041	1.71	1.67	8.9	0.061	0.467	0.504	0.007	0.35
	28	189.2	0.056	1.79	1.60	9.6	0.077	0.550	0.442	0.007	0.29
	29	178.6	0.052	1.78	1.64	8.9	0.067	0.505	0.536	0.006	0.35
	30	169.7	0.039	1.76	1.54	8.3	0.060	0.601	0.508	0.010	0.57
3F	31	200.4	0.044	1.79	1.84	9.7	0.072	0.553	0.640	0.011	0.40
	32	192.2	0.057	1.80	1.82	10.0	0.087	0.545	0.699	0.010	0.37
	33	188.9	0.043	1.77	1.71	9.1	0.068	0.511	0.493	0.008	0.31
	34	180.4	0.044	1.73	1.67	9.2	0.095	0.454	0.533	0.010	0.31
	35	197.9	0.045	1.80	1.85	8.9	0.075	0.578	0.609	0.010	0.38
4F	36	181.6	0.054	1.69	1.77	10.4	0.080	0.563	0.497	0.009	0.31
	37	159.4	0.051	1.74	1.42	7.3	0.057	0.454	0.411	0.007	0.56
	38	189.2	0.040	1.77	1.46	9.2	0.032	0.485	0.703	0.008	0.24
	39	171.2	0.031	1.67	1.59	7.9	0.044	0.451	0.472	0.012	0.47
	40	200.9	0.044	1.79	1.92	10.6	0.067	0.545	0.626	0.008	0.31

APPENDIX 4A - continued.

Absolute organ weights - individual values (g) for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	HEART	LUNGS & BR
1F	21	178.3	0.79	0.97
	22	194.8	0.93	1.32
	23	175.3	0.76	1.00
	24	180.5	0.74	0.97
	25	209.5	1.01	1.21
2F	26	178.6	0.77	1.26
	27	185.7	0.87	1.28
	28	189.2	0.93	1.08
	29	178.6	0.88	1.46
	30	169.7	0.75	1.03
3F	31	200.4	0.94	1.54
	32	192.2	0.91	1.27
	33	188.9	0.85	1.04
	34	180.4	0.87	1.19
	35	197.9	0.93	1.19
4F	36	181.6	0.89	1.27
	37	159.4	0.84	0.94
	38	189.2	1.12	1.51
	39	171.2	0.73	0.87
	40	200.9	0.85	1.37

APPENDIX 4B

Organ weights relative to bodyweight - individual values (%) for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	BRAIN	KIDNEYS	EPIDIDYMID	LIVER	SPLEEN	TESTES	THYMUS	THYROIDS+P
1M	1	222.0	0.0153	0.789	0.909	0.1014	5.89	0.3032	1.136	0.2685	0.0050
	2	226.8	0.0146	0.853	0.943	0.1459	4.95	0.2544	1.180	0.2831	0.0044
	3	240.8	0.0174	0.779	0.837	0.1100	5.92	0.3218	1.472	0.2272	0.0050
	4	234.6	0.0153	0.731	0.946	0.1394	5.81	0.2298	0.994	0.2217	0.0043
	5	212.2	0.0141	0.886	0.890	0.1018	4.96	0.2634	0.925	0.1800	0.0042
2M	6	247.7	0.0149	0.709	0.889	0.1147	5.52	0.2870	0.927	0.2321	0.0048
	7	255.3	0.0137	0.720	0.866	0.1187	5.94	0.3075	0.910	0.2096	0.0055
	8	246.5	0.0146	0.795	0.911	0.1465	5.04	0.2819	0.980	0.2966	0.0032
	9	243.6	0.0123	0.781	0.894	0.1318	5.00	0.2447	1.002	0.2878	0.0037
	10	259.1	0.0151	0.666	0.783	0.0899	6.30	0.2733	0.904	0.2918	0.0027
3M	11	227.1	0.0141	0.823	0.869	0.1185	5.35	0.2977	1.133	0.3065	0.0053
	12	243.5	0.0156	0.770	0.848	0.0924	5.35	0.3039	0.913	0.2012	0.0045
	13	220.6	0.0159	0.780	0.818	0.0925	4.85	0.2230	0.410	0.2815	0.0041
	14	225.5	0.0160	0.837	0.941	0.1308	5.15	0.3002	1.143	0.2723	0.0027
	15	231.5	0.0186	0.803	0.898	0.1343	4.98	0.3093	0.990	0.2229	0.0052
4M	16	229.1	0.0153	0.796	0.841	0.1152	5.48	0.2789	0.867	0.2702	0.0065
	17	262.3	0.0149	0.733	0.811	0.1022	4.83	0.2585	0.934	0.2207	0.0042
	18	223.1	0.0117	0.832	0.861	0.1219	5.19	0.2631	0.937	0.2797	0.0058
	19	208.4	0.0173	0.887	0.898	0.1392	5.42	0.2985	1.247	0.2673	0.0034
	20	218.0	0.0179	0.836	0.819	0.1413	5.14	0.2601	1.230	0.2674	0.0037

APPENDIX 4B - continued.

Organ weights relative to bodyweight - individual values (%) for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	HEART	LUNGS & BR
1M	1	222.0	0.415	0.514
	2	226.8	0.485	0.556
	3	240.8	0.438	0.501
	4	234.6	0.425	0.538
	5	212.2	0.414	0.578
2M	6	247.7	0.459	0.577
	7	255.3	0.413	0.491
	8	246.5	0.402	0.572
	9	243.6	0.492	0.534
	10	259.1	0.413	0.509
3M	11	227.1	0.488	0.512
	12	243.5	0.445	0.550
	13	220.6	0.583	0.575
	14	225.5	0.438	0.648
	15	231.5	0.426	0.487
4M	16	229.1	0.457	0.667
	17	262.3	0.418	0.606
	18	223.1	0.486	0.634
	19	208.4	0.454	0.728
	20	218.0	0.436	0.693

APPENDIX 4B - continued.

Organ weights relative to bodyweight - individual values (%) for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	BRAIN	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	THYROIDS+P	UTERUS + C
1F	21	178.3	0.0213	0.988	0.898	4.72	0.0342	0.2541	0.2664	0.0062	0.397
	22	194.8	0.0257	0.909	0.836	4.84	0.0252	0.3357	0.3537	0.0046	0.180
	23	175.3	0.0177	1.017	0.945	5.05	0.0371	0.3098	0.3120	0.0068	0.185
	24	180.5	0.0188	0.941	1.013	5.08	0.0410	0.2936	0.2454	0.0066	0.198
	25	209.5	0.0286	0.894	0.832	4.89	0.0353	0.4076	0.3437	0.0033	0.161
2F	26	178.6	0.0241	1.018	0.870	5.14	0.0274	0.2996	0.4446	0.0062	0.139
	27	185.7	0.0221	0.920	0.898	4.79	0.0328	0.2515	0.2714	0.0038	0.190
	28	189.2	0.0296	0.946	0.845	5.06	0.0407	0.2907	0.2336	0.0037	0.155
	29	178.6	0.0291	0.996	0.918	4.96	0.0375	0.2828	0.3001	0.0034	0.194
	30	169.7	0.0230	1.038	0.907	4.87	0.0354	0.3542	0.2994	0.0059	0.334
3F	31	200.4	0.0220	0.892	0.918	4.85	0.0359	0.2759	0.3194	0.0055	0.199
	32	192.2	0.0297	0.935	0.949	5.22	0.0453	0.2836	0.3637	0.0052	0.190
	33	188.9	0.0228	0.935	0.907	4.82	0.0360	0.2705	0.2610	0.0042	0.163
	34	180.4	0.0244	0.960	0.927	5.09	0.0527	0.2517	0.2955	0.0055	0.173
	35	197.9	0.0227	0.912	0.935	4.50	0.0379	0.2921	0.3077	0.0051	0.192
4F	36	181.6	0.0297	0.929	0.974	5.74	0.0441	0.3100	0.2737	0.0050	0.173
	37	159.4	0.0320	1.093	0.890	4.58	0.0358	0.2848	0.2578	0.0044	0.353
	38	189.2	0.0211	0.933	0.770	4.87	0.0169	0.2563	0.3716	0.0042	0.126
	39	171.2	0.0181	0.976	0.928	4.63	0.0257	0.2634	0.2757	0.0070	0.273
	40	200.9	0.0219	0.891	0.954	5.30	0.0333	0.2713	0.3116	0.0040	0.152

APPENDIX 4B - continued.

Organ weights relative to bodyweight - individual values (%) for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

Schedule number: GSB 060

GROUP	ANIMAL	TERMINAL BODY WT (g)	HEART	LUNGS & BR
1F	21	178.3	0.444	0.543
	22	194.8	0.479	0.679
	23	175.3	0.434	0.568
	24	180.5	0.408	0.538
	25	209.5	0.481	0.578
2F	26	178.6	0.431	0.703
	27	185.7	0.469	0.687
	28	189.2	0.491	0.572
	29	178.6	0.496	0.817
	30	169.7	0.441	0.608
3F	31	200.4	0.467	0.770
	32	192.2	0.475	0.663
	33	188.9	0.452	0.550
	34	180.4	0.481	0.660
	35	197.9	0.471	0.602
4F	36	181.6	0.489	0.699
	37	159.4	0.524	0.590
	38	189.2	0.590	0.800
	39	171.2	0.426	0.507
	40	200.9	0.421	0.682

APPENDIX 5

Macropathology - individual findings for animals killed after 7 days of treatment.

```
Group                :    1      2       3      4                                                    Printed: 02-SEP-99
Compound             : Control     ---- ARO-1 ----                                                      Page: 1
Dosage (mg/kg/day)   :    0      500    1500    5000
                                                                                                  Schedule number: GSB 060
-------------------------------------------------------------------------------------------------------------------------------
ANIMAL NUMBER: 0001           SEX: MALE           DOSE GROUP: 1       SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99      STUDY DAY OF DEATH: 8       STUDY WEEK OF DEATH: 2        TERMINAL BODY WEIGHT:   222.0 GRAMS
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    *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 2
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0002 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 226.8 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0003 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 240.8 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	:	1	2	3	4
Compound	:	Control	----	ARO-1	----
Dosage (mg/kg/day)	:	0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0004 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 234.6 GRAMS

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
THYROIDS (TD)		
	-APPEAR SMALL	-LEFT. WT. 0.002G.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0005 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 212.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0006 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 247.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0007	SEX: MALE	DOSE GROUP: 2	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE	
DATE OF DEATH: 26-JAN-99	STUDY DAY OF DEATH: 8	STUDY WEEK OF DEATH: 2	TERMINAL BODY WEIGHT:	255.3 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0008 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 246.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0009 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 243.6 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0010 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 259.1 GRAMS

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
L N THYMIC (LT)		
	-CYSTIC	-OCCASIONAL NODES.
SKIN (SK0)		
	-ENCRUSTATION(S)	-DARK AREA, 3X2MM, ON LEFT UPPER DORSAL THORAX.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0011 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 227.1 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0012 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 243.5 GRAMS

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
KIDNEYS (KI)		
	-HYDRONEPHROSIS	-LEFT. SLIGHT. WT. 1.013G.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	:	1	2	3	4
Compound	:	Control	----	ARO-1	----
Dosage (mg/kg/day)	:	0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0013 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 220.6 GRAMS

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
EPIDIDYMIDES (ED)	-APPEAR SMALL	-LEFT. WT. 0.095G. RIGHT. WT. 0.109G.
TESTES (TS)	-APPEAR SMALL	-LEFT. WT. 0.457G. RIGHT. WT. 0.447G.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	:	1	2	3	4
Compound	:	Control	----	ARO-1	----
Dosage (mg/kg/day)	:	0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0014 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 225.5 GRAMS

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
SUBMANDIB SL.GL. (SA)		
	-AREA(S) OF CHANGE	-RIGHT. POORLY DEFINED, DARK AREA.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0015 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 231.5 GRAMS

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
TRACHEA (TR)		
	-ABNORMAL CONTENTS	-CONTAINS DARK FLUID.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0016 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 229.1 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	
Compound	: Control	----	ARO-1	----	
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0017 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 262.3 GRAMS

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
PITUITARY (PI)		
	-CYSTIC	-CLEAR CYST, 2MM DIA., ON DORSAL SURFACE.
		NECROPSY NOTE: >IN SITU.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0018 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 223.1 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 19
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0019 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 208.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 20
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0020	SEX: MALE	DOSE GROUP: 4	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99	STUDY DAY OF DEATH: 8	STUDY WEEK OF DEATH: 2	TERMINAL BODY WEIGHT: 218.0 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0021 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 178.3 GRAMS

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
UTERUS (UT)		
	-FLUID DISTENSION	-<4MM DIA.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0022 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 194.8 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 23
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0023 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 175.3 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 24
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0024 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 180.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0025	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99	STUDY DAY OF DEATH: 8	STUDY WEEK OF DEATH: 2	TERMINAL BODY WEIGHT: 209.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group : 1 2 3 4
Compound : Control ---- ARO-1 ----
Dosage (mg/kg/day) : 0 500 1500 5000

Schedule number: GSB 060

ANIMAL NUMBER: 0026 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 178.6 GRAMS

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
KIDNEYS (KI)		
	-HYDRONEPHROSIS	-RIGHT. SLIGHT. WT. 0.788G.

APPENDIX 5 · continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0027 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 185.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 28
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0028 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 189.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARD-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0029 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 178.6 GRAMS

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
KIDNEYS (KI)		
	-HYDRONEPHROSIS	-RIGHT. SLIGHT. WT. 0.798G.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0030 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 169.7 GRAMS

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
UTERUS (UT)		
	-FLUID DISTENSION	-<4MM DIA.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0031 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 200.4 GRAMS

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
KIDNEYS (KI)		
	-HYDRONEPHROSIS	-RIGHT. SLIGHT. WT. 0.922G.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0032 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 192.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0033 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 188.9 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0034 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 180.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0035 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 197.9 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 36
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0036 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 181.6 GRAMS

* * * GROSS PATHOLOGY OBSERVATIONS * * *

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
KIDNEYS (KI)		
	-HYDRONEPHROSIS	-RIGHT. MODERATE. WT. 0.899G.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0037 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 159.4 GRAMS

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
THYROIDS (TD)	-APPEAR SMALL	-LEFT. WT. 0.001G.
UTERUS (UT)	-FLUID DISTENSION	-<3MM DIA.

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4	Printed: 02-SEP-99
Compound	: Control	----	ARO-1	----	Page: 38
Dosage (mg/kg/day)	: 0	500	1500	5000	

Schedule number: GSB 060

ANIMAL NUMBER: 0038 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 189.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control		---- ARO-1 ----	
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0039 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 171.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology - individual findings for animals killed after 7 days of treatment.

Group	: 1	2	3	4
Compound	: Control	----	ARO-1	----
Dosage (mg/kg/day)	: 0	500	1500	5000

Schedule number: GSB 060

ANIMAL NUMBER: 0040 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 26-JAN-99 STUDY DAY OF DEATH: 8 STUDY WEEK OF DEATH: 2 TERMINAL BODY WEIGHT: 200.9 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

Huntingdon Life Sciences

PROTOCOL

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Sponsor

Gist-Brocades BV
CT&S/REG
PO Box 1
Wateringseweg 1
NL-2600 MA Delft
THE NETHERLANDS

Research Laboratory

Huntingdon Life Sciences Ltd
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Total number of pages: 18

Final Protocol

CONTACT DETAILS

Sponsor's Monitoring Scientist : Mrs R. Hempenius.

Huntingdon Life Sciences

PROTOCOL APPROVAL

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

(b) (6)

4 January 1999

S. Patel, B.Sc., C.Biol., M.I.Biol. — Date
Study Director,
Huntingdon Life Sciences Ltd.

The signature of the Study Director confirms this protocol as the working document for the study. Any changes made subsequent to the date of the Study Director's signature will be documented in formal amendments.

(b) (6)

4 January '99

P. Aughton, B.Sc., D.A.B.T., Dip.R.C.Path (Tox)., C.Biol., M.I.Biol. — Date
Management,
Huntingdon Life Sciences Ltd.

(b) (6)

13 January '99

Mrs R. Hempenius — Date
Sponsor,
Gist-Brocades BV.

Please sign both copies of this page, retain one for your records and return one to the Study Director at Huntingdon Life Sciences.

Huntingdon Life Sciences

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Enquiry Number: 16098C

Number of pages for internal distribution: 15

This working document is approved for circulation and use:

....

Study Director Date

Primary location of study

Eye Research Centre
Eye
Suffolk

Building Number: 4

All procedures to be performed at the above site unless otherwise detailed below.

Final Protocol

CONTENTS

1. INTRODUCTION

Management of study

Study Director	:	S. Patel.
Monitoring Toxicologist	:	P. Aughton.
In the temporary absence of the Study Director, the scientific responsibilities will be taken over by the Monitoring Toxicologist; other items of routine study management should be referred to the following person in the first instance.	:	S. Cooper.

Objective

Assessment of systemic toxic potential in a 1 week oral gavage study in CD Rats.

Good Laboratory Practice

The study will be conducted in compliance with principles of Good Laboratory Practice Standards as set forth in:

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29).

No specific study-related Quality Assurance procedures or analysis of dose form will be performed.

Animal model	:	CD Rats, accepted by regulatory agencies, background data available.
Route	:	Oral Gavage, to simulate the conditions of clinical administration.

Treatment groups and dosages

Group	:	1	2	3	4
Compound	:	Control	----------	ARO-1	----------
Dosage (mg/kg/day)	:	0	500	1500	5000

2. STUDY SCHEDULE AND STRUCTURE

2.1. Duration of treatment

Minimum period : 1 week.

The treatment period may be extended, with the Sponsor's consent, in order to investigate any equivocal or progressive effects; documented in an amendment to protocol.

Treatment will also continue throughout the necropsy period. The serial observations will be recorded at appropriate intervals (Section 4.3). Data for any additional complete weeks before commencement of necropsies will be included in the final report.

2.2. Scheduled time plan

(to be up-dated as required in an amendment to protocol)

Sample of (ARO-1) arrived	:	23 December 1998	
Animals to arrive	:	6 January 1999	
Treatment to commence	:	19 January 1999	
Terminal sacrifice to commence	:	26 January 1999	(estimated)
Draft report to be issued	:	w/b 15 February 1999	(estimated)

2.3. Identity of treatment groups

(to be selected from 50 animals ordered)

Group	Treatment	Dosage (mg/kg/day)* #	Number of animals	
			Male	Female
1	Control	0	5	5
2	ARO-1	500	5	5
3	ARO-1	1500	5	5
4	ARO-1	5000	5	5

\# Expressed in terms of the test substance as supplied.
* Dosage (mg/kg/day) selected by the Sponsor.

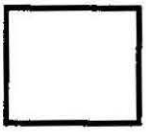
Group	Cage numbers		Animal numbers	
	Male	Female	Male	Female
1	1	5	1-5	21-25
2	2	6	6-10	26-30
3	3	7	11-15	31-35
4	4	8	16-20	36-40

3. TEST SUBSTANCE AND FORMULATION

In order for Huntingdon Life Sciences to comply with the Health and Safety at Work etc. Act 1974, and the Control of Substances Hazardous to Health Regulations 1994, it is a condition of undertaking the study that the Sponsor shall provide Huntingdon Life Sciences with all information available to it regarding known or potential hazards associated with the handling and use of any substance supplied by the Sponsor to Huntingdon Life Sciences. The Sponsor shall also comply with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed Huntingdon Life Sciences test substance data sheet must be received by Safety Management Services at Huntingdon Life Sciences before the test substance can be handled in the laboratory. At the discretion of Safety Management Services at Huntingdon Life Sciences, other documentation containing the equivalent information may be acceptable.

Information received will be used to set the Huntingdon Life Sciences Hazard Class, which determines safety precautions taken in the workplace.

Huntingdon Life Sciences Hazard Class: 

3.1. Test substance

Sponsor's identification	:	Enzyme preparation from *Aspergillus niger* (ARO-1).
Storage conditions	:	Deep-frozen (approximately -20°C), and protected from light.
Sponsor's responsibilities	:	Documentation of methods of synthesis, fabrication or derivation. Stability data. Certificate of analysis.
Certificate of analysis details	:	Test substance identity. Batch number. Purity. Composition. Other appropriate characteristics. Current expiry date.

3.2. Formulation

Treatment		
Group 1, Control	:	Vehicle.
Group 2	:	*Aspergillus niger* (ARO-1); 50 mg/ml.
Group 3	:	*Aspergillus niger* (ARO-1); 150 mg/ml.
Group 4	:	*Aspergillus niger* (ARO-1); 500 mg/ml.
Conversion factor	:	The test substance will be used as supplied.
Vehicle	:	Water obtained be reverse osmosis.
Method of preparation	:	Will be documented in the study data and included in the final report.
Frequency of preparation	:	Will depend upon the availability of supporting stability data. Where sufficient stability data is available, batches will cover one week of dosing and may be prepared up to three days in advance of the first day of dosing. Where stability data does not support this length of use period, a more frequent mixing regime will be initiated.

3.3. Quality control of dosage form

Liquid formulation	:	Before commencement of treatment, the suitability of the proposed mixing procedures will be determined by visual assessment.
Assay sampling	:	For compliance with international GLP regulations it may be necessary to analyse samples of test formulations to confirm homogeneity, stability and achieved concentration. However no such analyses will be undertaken without the instruction of the Sponsor. Samples of test formulations will be taken, stored or sent to the Sponsor if requested before commencement of treatment.

4. ANIMAL MANAGEMENT

4.1. Animals - supply, acclimatisation and allocation

4.1.1. Animals

Species	:	Rat.
Strain	:	Crl:CD® BR.
Age ordered	:	28 ± 2 days.
Weight range ordered	:	11 g/sex.
Supplier	:	Charles River (UK) Limited.

4.1.2. Acclimatisation

Duration	:	At least 7 days before commencement of treatment.
Husbandry conditions	:	Refer to Section 4.2.

4.1.3. Allocation to treatment groups

Allocation	:	On arrival.
Method	:	Random.
Cage distribution	:	To equalise environmental influences between groups.

4.1.4. Identification

Numbering	:	Unique for each animal within study.
Method	:	Tail tattoo.
Cage labels	:	Uniquely identifying the occupants.

4.1.5. Animal replacement

10 spare animals will be ordered to replace any individuals rejected during the acclimatisation period.

Replacement before treatment	:	Ill-health. Abnormalities. Bodyweight range extremes.
Replacement during treatment	:	None scheduled.

4.2. Animals - housing, diet and water supply

4.2.1. Environmental control

Rodent facility	:	Full barrier - to minimise entry of external biological and chemical agents.
Air supply	:	Filtered, not recirculated.
Temperature	:	Target range: 19-23°C.
Relative humidity	:	Target range: 40-70%.

Monitored continuously or daily. Excursions outside these ranges documented in the study data.

Lighting	:	12 hours light : 12 hours dark.
Alarm systems	:	Activated on ventilation failure and when temperature/humidity limits exceeded.
Electricity supply	:	Public supply with automatic stand-by generators.

4.2.2. Animal accommodation

Animals per cage	:	Five of the same sex, unless reduced by mortality or isolation.
Cage material	:	Polypropylene or stainless steel.
Cage flooring	:	Stainless steel grid.

The cages will be suspended above absorbent paper. The latter will be changed at appropriate intervals each week; cages, cage-trays, food hoppers and water bottles will be changed at appropriate intervals. Precise details of caging will be included in the final report.

4.2.3. Diet and water supply

Copies of all certificates of analysis are stored in the archives.

Diet supply

Diet name	:	Rat and Mouse No. 1 Maintenance Diet.
Diet type	:	Pelleted diet.
Availability	:	Non-restricted.
Certification	:	Before delivery each batch of diet is analysed by the supplier for various nutritional components and chemical and microbiological contaminants. Supplier's analytical certificates are scrutinised and approved before any batch of diet is released for use.

This diet contains no added antibiotic or other chemotherapeutic or prophylactic agent.

Water supply

Supply	:	Public drinking water.
Regulatory agency	:	U.K. Department of the Environment.
Availability	:	Non-restricted via polyethylene or polycarbonate bottles with sipper tubes.
Certification	:	Certificates of analysis are routinely received from the supplier.

4.2.4. Contaminants assay

It is the Sponsor's responsibility to advise Huntingdon Life Sciences of any specific contaminants likely to prejudice the outcome of the study. Analyses for such contaminants may be performed if requested by the Sponsor.

4.3. Animals - procedures

Day numbers, where quoted, may be varied by not more than 2 days. Examinations scheduled for before termination of treatment will be undertaken during the last scheduled week of treatment unless otherwise specified. The precise times of all examinations will be included in the final report.

4.3.1. Administration

Route	:	Oral gavage.
Treated at	:	Constant dosages in mg/kg/day.
Volume dosage	:	10 ml/kg/day.
Individual dose volume	:	Calculated from the most recently recorded scheduled bodyweight.
Controls (Group 1)	:	Vehicle at the same volume dosage as treated groups.
Frequency	:	Once daily at approximately the same time each day.
Sequence	:	By group.
Formulation	:	A daily record of the usage of formulation will be maintained based on weights. This balance is compared with the expected usage as a check of correct administration. Suspensions are stirred using a magnetic stirrer before and throughout the dosing procedure.

4.3.2. Clinical observations

Animals and their cages	:	Inspected at least twice daily for evidence of reaction to treatment or ill-health.
Deviations from normal recorded at the time in respect of	:	Nature and severity. Date and time of onset. Duration and progress of the observed condition.
Physical examination	:	Once.

In addition detailed observations will be made in association with dosing according to the following schedule and frequency:

Minimum schedule	:	Week 1 - daily.
Frequency	:	1. Pre-dose observation. 2. As each animal is returned to its home cage. 3. At the end of dosing each group. 4. Between 1 and 2 hours after completion of dosing all groups. 5. As late as possible in the working day.

The above schedule will be amended, as necessary, in the light of signs observed.

During the acclimatisation period, observations of the animals and their cages will be recorded at least once per day.

4.3.3. Mortality

Debilitated animals	:	Observed carefully, may be isolated to prevent cannibalism.
Premature sacrifice	:	Animals may be killed on humane grounds or if considered *in extremis*.
Animals found dead, killed *in extremis* or on humane grounds	:	A necropsy is performed as soon as possible. Animals found outside the normal workday will be preserved in a refrigerator (approximately 4°C) provided for this purpose.

4.3.4. Bodyweight

Bodyweight recording	:	Day that treatment commences. Twice weekly. At necropsy.

More frequent weighing may be performed to aid the monitoring of the condition of animals displaying ill-health. These data will be retained in the archives.

4.3.5. Food consumption

Food consumption recording	:	Week 1.
Food supplied	:	At intervals each week.
Food spilled	:	Recorded at cage cleaning.
Food remaining	:	Recorded at end of study week.

4.4. Animals - termination

All animals will be subject to terminal investigations (Section 5). The sequence in which the animals are killed after completion of treatment will allow satisfactory inter-group comparison.

5. NECROPSY AND HISTOLOGY

5.1. Method of kill

Method	:	Carbon dioxide.
Sequence	:	To allow satisfactory inter-group comparison.

5.2. Macroscopic Pathology

(Table 1)

Complete	:	All animals.
Checks	:	Retained tissues.
Photography	:	Unusual or suspected treatment-related findings; at the discretion of the necropsy supervisor or Study Director.
Special requirements	:	Retain lymph nodes adjacent to masses (where appropriate).

5.3. Organ weights

(Table 1)

Data collection	:	For bilateral organs, left and right organs will be weighed together unless otherwise specified on the Pathology Procedures Table.
Data presentation	:	Absolute. Adjusted for terminal bodyweight.

5.4. Fixation

(Table 1)

Standard	:	10% Neutral Buffered Formalin.
Others	:	Testes and epididymides: Initially in Bouin's fluid. Eyes: In Davidson's fluid.

5.5. Histology and light microscopy

(Optional)

Histological processing and microscopic examination of the retained tissues will only be performed, and documented in an amendment to the protocol, if requested by the Sponsor.

TABLE 1 - Pathology procedures

Tissue	Weigh	Fix
Abnormalities		*
Adrenals	*	*
Brain	*	*
Caecum		*
Colon		*
Duodenum		*
Epididymides	*	*
Head		b)
Heart	*	*
Ileum		*
Jejunum		*
Kidneys	*	*
Liver	*	*
Lungs	*	*
Lymph nodes - mandibular		*
- mesenteric		*
Oesophagus		*
Ovaries	*	*
Prostate		*
Rectum		*
Sciatic nerve		*
Spinal cord		*
Spleen	*	*
Stomach		*
Testes	*	*
Thymus	*	*
Thyroid with parathyroids	a)	*
Trachea		*
Urinary bladder		*
Uterus with cervix	*	*

a) Weigh after fixation.
b) Including nasal cavity, paranasal sinuses and nasopharynx.
* Organs weighed or samples fixed.

6. DATA TREATMENT

6.1. Food conversion efficiency

The group mean food conversion efficiency of each sex, expressed as bodyweight gain per unit of food consumed as a percentage, will be calculated for each week of the study.

6.2. Statistical analysis

Data-types

The following data types will be analysed at each timepoint separately:-

bodyweight, using gains over appropriate study periods.
organ weights, both absolute and adjusted for terminal bodyweight.
pathological findings, for the number of animals with and without each finding.

Methods

For categorical data, the proportion of animals will be analysed using Fisher's Exact test for each treated group versus the control.

For continuous data, Bartlett's test will first be applied to test the homogeneity of variance between the groups. Using tests dependent on the outcome of Bartlett's test, treated groups will then be compared with the control group, incorporating adjustment for multiple comparisons where necessary.

7. REPORTING

Study progress	:	Periodic verbal and written updates on study progress will be provided by the Study Director. Status reports will be sent during the in-life phase.
Draft final report	:	For review by the Sponsor.
Authorised final report	:	After approval from the Sponsor.

Routinely reports are supplied on A4 paper. The following numbers of reports are supplied.

Type of report	Printing	Number of copies	
		Bound	Unbound
Draft report	Double-sided	0	2
Authorised final	Double-sided	1	0
	Single-sided	0	1
Photographic report (if any)	Single-sided	1	0

Any additions or corrections to an authorised report will be documented as a formal addendum/amendment to the final report.

8. QUALITY ASSURANCE AND ARCHIVING PROCEDURES

8.1. Quality Assurance

No formal study-based Quality Assurance procedures will be performed on this study. These may be included if requested by the Sponsor.

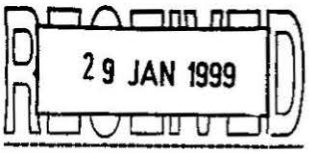
8.2. Archives

All experimental data arising from the study (including documentary raw data, specimens, records, other materials; collectively defined as the "materials") will remain the property of the Sponsor.

Huntingdon Life Sciences shall retain the materials in its archive for a period of 10 years from the date of issue of the final report. After such time, the Sponsor will be contacted and their advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Study Number : GSB/060
Protocol Amendment Number : 1

Huntingdon Life Sciences



ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Total number of pages: 6

Number of pages for internal distribution: 5

Study Director : S. Patel, B.Sc., C.Biol., M.I.Biol.

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

FIRST AMENDMENT APPROVAL

For Huntingdon Life Sciences Ltd

Authorised by: (b) (6) Date: 18 January 1999
(Study Director)

For the Sponsor (b) (6)

Approved by: Date: 25 January 1999

Huntingdon Life Sciences

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Reason for amendment	:	The number of pages of the entire protocol and those to be distributed is corrected.
	:	Section 3: The hazard class and the frequency of preparation of formulations is included.

Amendments

Huntingdon Life Sciences

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Enquiry Number: 16098C

Number of pages for internal distribution: ~~15~~ 14

This working document is approved for circulation and use:

.....

Study Director

.....

Date

Primary location of study

Eye Research Centre
Eye
Suffolk

Building Number: 4

All procedures to be performed at the above site unless otherwise detailed below.

3. TEST SUBSTANCE AND FORMULATION

In order for Huntingdon Life Sciences to comply with the Health and Safety at Work etc. Act 1974, and the Control of Substances Hazardous to Health Regulations 1994, it is a condition of undertaking the study that the Sponsor shall provide Huntingdon Life Sciences with all information available to it regarding known or potential hazards associated with the handling and use of any substance supplied by the Sponsor to Huntingdon Life Sciences. The Sponsor shall also comply with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed Huntingdon Life Sciences test substance data sheet must be received by Safety Management Services at Huntingdon Life Sciences before the test substance can be handled in the laboratory. At the discretion of Safety Management Services at Huntingdon Life Sciences, other documentation containing the equivalent information may be acceptable.

Information received will be used to set the Huntingdon Life Sciences Hazard Class, which determines safety precautions taken in the workplace.

Huntingdon Life Sciences Hazard Class: 2

3.2 Formulation

Treatment		
Group 1, Control	:	Vehicle.
Group 2	:	*Aspergillus niger* (ARO-1); 50 mg/ml.
Group 3	:	*Aspergillus niger* (ARO-1); 150 mg/ml.
Group 4	:	*Aspergillus niger* (ARO-1); 500 mg/ml.
Conversion factor	:	The test substance will be used as supplied.
Vehicle	:	Water obtained be reverse osmosis.
Method of preparation	:	Will be documented in the study data and included in the final report.

Frequency of preparation : ~~Will depend upon the availability of supporting stability data. Where sufficient stability data is available, batches will cover one week of dosing and may be prepared up to three days in advance of the first day of dosing. Where stability data does not support this length of use period, a more frequent mixing regime will be initiated~~.
Weekly
Formulations will be divided into daily aliquots and stored refrigerated (approximately 4°C) before use.

Huntingdon Life Sciences

PROTOCOL

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Sponsor

Gist-Brocades BV
CT&S/REG
PO Box 1
Wateringseweg 1
NL-2600 MA Delft
THE NETHERLANDS

Research Laboratory

Huntingdon Life Sciences
Ltd
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Total number of pages: ~~18~~ 17

Final Protocol

Study Number : GSB060
Protocol Amendment Number : 2

RECEIVED 09 FEB 1999

Huntingdon Life Sciences

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

PRELIMINARY TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO CD RATS FOR

1 WEEK

Study Director (original) : S. Patel, B.Sc., C.Biol., M.I.Biol.

Study Director (replacement) : S. Cooper, B.Sc. C.Biol., M.I.Biol.

This amendment formally registers the assignment of a replacement Study Director. The signature of the replacement Study Director approves the implementation of this amendment to protocol. Any changes to the study design after the date of this approval signature will be documented in a further formal amendment.

Reason for amendment : The original Study Director (S. Patel) is leaving employment with Huntingdon Life Sciences.

Declaration

Original Study Director : I am satisfied with the conduct of the study to date.

Signature: (b) (6) Date: 29 January 1999

Replacement Study Director : I am satisfied with the conduct of the study to date. From the date of my signature on this amendment I assume the responsibilities of the Study Director.

Signature: (b) (6) Date: 29 January 1999

SECOND AMENDMENT APPROVAL

For Huntingdon L (b) (6)

Authorised by: Date: 29 January 1999
(Replacemen (b) (6)

Released by: Date: 29 January '99

For Sponsor (b) (6)

Approved by: Date: 4 February 1999

Annex 8

Beta-glucosidase oral 28-day toxicity study in rats

DSM R&D-archives Delft

P.O. Box 1,
2600 MA Delft
The Netherlands

DSM

Report number: 15.838

Date: 3-2-2000

ENZYME PREPARATION FROM *ASPERGILLUS NIGER* (ARO-1) TOXICITY STUDY BY ORAL GAVAGE ADMINSTRATION TO CD RATS FOR 4 WEEKS

CRO-REPORT NO. GSB061/993953

Author(s): S. Cooper

Department: Huntingdon Life Sciences Ltd.

Experimental work:

Keywords: Toxicology
Subacute-tox
Oral
Beta-D-glucosidase
Aspergillus-niger
ARO-1
GLP-9708

Mailing list

R.A. Hempenius Archiefrapportage (2x)	

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ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO

CD RATS FOR 4 WEEKS

Sponsor

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Draft: 18 August 1999
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PROTOCOL

Huntingdon Life Sciences

Working for a better future

COMPLIANCE WITH GOOD LABORATORY PRACTICE

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

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The study described in this report was conducted in compliance with the following Good Laboratory Practice standards. I consider the data generated by Huntingdon Life Sciences during the course of this study to be valid and that the final report fully and accurately reflects this raw data.

United Kingdom Good Laboratory Practice Regulations, 1997 (Statutory Instrument No 654) and, from 14 December 1999, United Kingdom Good Laboratory Practice Regulations 1999 (Statutory Instrument No. 3106).

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29), and from 1 May 1999 EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The information contained in the Certificates of Analysis in Appendix 1 is provided by the Sponsor and GLP Compliance of these data is the responsibility of the Sponsor.

(b) (6)

S. Cooper, B.Sc., C.Biol., M.I.Biol.
Study Director
Huntingdon Life Sciences Ltd.

24 January 2000
Date

Huntingdon Life Sciences

QUALITY ASSURANCE STATEMENT

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

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CD RATS FOR 4 WEEKS

The following have been inspected or audited in relation to this study

Study Phase	**Date of Inspection**	**Date of Reporting**
Protocol Audit	23 March 1999	23 March 1999
Study Based Inspections		
Dose Preparation	06 April 1999	07 April 1999
Dose Administration	07 April 1999	08 April 1999
Necropsy	05 May 1999	05 May 1999
Report Audit	15 November 1999	15 November 1999

Protocol Audit: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Study based inspections: Inspections and audits of phases of this study were conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections of other routine and repetitive procedures employed on this type of study were carried out. These were promptly reported to appropriate Company Management.

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.

(b) (6)

24 January 2000

H. Comb, B.Sc.
Group Manager
Department of Quality Assurance
Huntingdon Life Sciences Ltd.

Date

Huntingdon Life Sciences

RESPONSIBLE PERSONNEL

ENZYME PREPARATION FROM ASPERGILLUS NIGER (ARO-1)

TOXICITY STUDY BY

ORAL GAVAGE ADMINISTRATION TO

CD RATS FOR 4 WEEKS

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Clinical Pathology Manager

Blood Chemistry

D. Crook, B.Sc., Ph.D.
Head of Data Quality
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PATHOLOGY

K.C.D. Liu, B.Sc., M.Sc., Ph.D.
Staff Pathologist

SUMMARY

Groups of ten male and ten female CD rats received Enzyme preparation from *Aspergillus niger* (ARO-1), by oral gavage, at dosages of 500, 1500 or 5000 mg/kg/day for four weeks. A similarly constituted Control group received vehicle alone.

No animals died and there were no signs related to treatment.

Bodyweights, food intake and food conversion efficiency were unaffected by treatment.

There were no ophthalmoscopic lesions which were considered to be related to treatment.

There were no treatment related haematological findings.

Slightly low plasma phosphorus concentrations were observed in animals receiving 5000 mg/kg/day and in males receiving 1500 mg/kg/day.

Organ weights were not affected by treatment and there were no macroscopic findings noted which were considered to be related to the administration of Enzyme preparation from *Aspergillus niger* (ARO-1).

Microscopic examination revealed a higher incidence of inflammatory cell infiltrate in the lamina propria of the caecum in treated females when compared with the Controls. The number of animals affected in each group were three, two and seven for females which had received 500, 1500 or 5000 mg/kg/day respectively.

It is concluded that treatment of CD rats with Enzyme preparation from *Aspergillus niger* (ARO-1) at dosages of 500, 1500 and 5000 mg/kg/day resulted in some minor changes. All changes were considered to be of no toxicological significance. The dosage of 5000 mg/kg/day is considered to be the No-Observed-Adverse-Effect-Level (NOAEL) in this study.

INTRODUCTION

Objective

The objective of this study was to assess the systemic toxic potential of Enzyme preparation from *Aspergillus niger* (ARO-1) during its repeated daily oral administration to CD rats for four weeks. The study was designed to meet the requirements of the Food and Drug Administration for the USA.

Justification for the test system

The rat was chosen because of its acceptance as a predictor of toxic change in man and the requirement for a rodent species by regulatory agencies. The CD strain was used because of the historical control data available in this laboratory.

Justification for the treatment regimen

The oral route (gavage) was selected to simulate the conditions of human exposure during use of the test substance.

Dosages of 500, 1500 and 5000 mg/kg/day were based on the results from a preliminary study performed at these laboratories (Report No. GSB060/980021). This study concluded that dosages up to 5000 mg/kg/day did not result in any changes which were considered to be of toxicological significance.

The duration of treatment was selected to accord with regulatory requirements.

Study organisation

Testing facilities:

The principal laboratory was:

Huntingdon Life Sciences Ltd
Eye
Suffolk
IP23 7PX
England

The analyses described in the 'Test Material' and 'Quality Control of Dosage Form' sections of this report were performed by:

Gist-Brocades BV
Department DSM-R/PAC/ARS
A Fleminglaan 1
2613 AX Delft
The Netherlands

The data from the analyses of the dose formulations are reported separately by the Sponsor.

Study timing:

Animals arrived (Experimental start)	:	24 March 1999
Treatment commenced	:	7 April 1999
Necropsy completed	:	6 May 1999
Experimental finish (Issue of histopathological report)	:	10 August 1999

Archives

Following completion of this study all raw data, specimens and samples, except those generated or used during any Sponsor's or supplier's analysis, were stored in the archives of Huntingdon Life Sciences. A copy of the final report was also retained.

MATERIALS AND METHODS

DESIGN CONDITIONS

Animals

A total of 45 male and 45 female rats of the CD strain, 26 to 30 days of age, were obtained from Charles River (UK) Limited, Margate, Kent, England. The males used on the study weighed 154 to 195 g on the day that treatment commenced; females weighed 144 to 186 g.

Identification

After random allocation to groups each animal was assigned a number and identified uniquely within the study by a tail tattoo. Tattoos were checked at regular intervals.

Acclimatisation and age at commencement

The animals were allowed to acclimatise to the conditions described below for 14 days before commencement of treatment. They were 40 to 44 days of age when treatment started.

Environmental control

Animals were housed inside a barriered rodent facility.

Each animal room was kept at positive pressure with respect to the outside by its own supply of filtered fresh air which was passed to atmosphere and not re-circulated. Target values within the study room were 21°C for temperature (acceptable limits 19-25°C), 55% for relative humidity (acceptable limits 40–70%) and at least 15 air changes per hour. Lighting was controlled to provide a 12-hour light : 12-hour dark cycle.

The facility was designed and operated to minimise the entry of external biological and chemical agents and to minimise the transference of such agents between rooms. Before each study the room was cleaned and disinfected with a bactericide.

Access was limited to authorised personnel who were required to shower and change into clean protective clothing. Where practicable, materials and equipment entered the facility through an autoclave or a chamber in which their external surfaces were treated with a bactericide.

Alarms were available to be activated if there was any failure of the ventilation system, or temperature limits were exceeded.

Periodic checks were made on the number of air changes in the animal rooms. Temperature and humidity were monitored by continuous recordings. On Days 8 and 9, the humidity was found to be below the target range. There was no outward sign in any animal in response to the low humidity and, consequently, these minor deviations were not considered to have had any adverse impact on the study. Since these data show that there were no other significant deviations from target values they are not presented.

A stand-by electricity supply was available to be automatically brought into operation should the public supply fail.

Animal accommodation

The animals were housed five of one sex per cage, in RS Biotech cages from RS Biotech, Tower Works, Finedon, Northamptonshire, England, which were made of a stainless steel body with a stainless steel mesh lid and floor. The cages were suspended above absorbent paper which was changed at appropriate intervals. Cages, cage-trays, food hoppers and water bottles were also changed at appropriate intervals.

Diet and water supply

The animals were allowed free access, except overnight before routine blood sampling, to an expanded rodent diet, Rat and Mouse No. 1 Maintenance Diet from Special Diets Services Ltd., Witham, Essex, England. This diet contained no added antibiotic or other chemotherapeutic or prophylactic agent. Weighed amounts of diet were provided at intervals during each week to each cage.

At the end of each treatment week the weight of uneaten food was recorded. The uneaten diet may have been included in that returned to the cage, after appropriate measurement.

Water taken from the public supply (Essex and Suffolk Water Company, Chelmsford, Essex, England), was freely available, via polycarbonate bottles fitted with sipper tubes.

Quality control of diet and water

Each batch of diet was routinely sampled for analysis by the supplier for various nutritional components and chemical and microbiological contaminants. Supplier's analytical certificates were scrutinised and approved before any batch of diet was released for use.

The quality of the water supply is governed by regulations published by the Department of the Environment. Certificates of analysis were routinely received from the supplier.

Since the results of these various analyses did not provide evidence of contamination that might have prejudiced the study they are not presented.

No other specific contaminants that were likely to have been present in the diet or water were analysed, as none that may have interfered with or prejudiced the outcome of the study was known.

Allocation to treatment groups

On arrival, animals were non-selectively assigned to cages and treatment groups.

All animals were weighed during the acclimatisation period. Four males were replaced prior to the start of the study due to non-resolving ophthalmic lesions.

As far as was practicable, the distribution of animals in the room was designed to minimise the effect of any spatially variable component of the environment. The distribution is shown in Figure 1.

Composition and identity of treatment groups

Animals were assigned to the groups as follows:

Group	Treatment	Dosage (mg/kg/day)	Cage numbers		Animal numbers	
			Male	Female	Male	Female
1	Control	0	1-2	9-10	1-10	41-50
2	Enzyme preparation from *Aspergillus niger* (ARO-1)	500	3-4	11-12	11-20	51-60
3	Enzyme preparation from *Aspergillus niger* (ARO-1)	1500	5-6	13-14	21-30	61-70
4	Enzyme preparation from *Aspergillus niger* (ARO-1)	5000	7-8	15-16	31-40	71-80

Cage labels, identifying the occupants by experiment, animal number, sex and treatment group, were colour-coded.

TREATMENT

Test material

A consignment of 3.15 kg of Enzyme preparation from *Aspergillus niger* (ARO-1) taken from batch no. RER 710 was received from the Sponsor in nine aliquots, at the Huntingdon Research Centre (HRC) on 23 December 1998 and these aliquots were subsequently transferred to Eye Research Centre (ERC) on 6 January 1999. It was a brown liquid (frozen). Three aliquots each weighed approximately 250 g and the remaining six each weighed approximately 400 g (this information was supplied by the Sponsor).

The test material was stored in a freezer (approximately -20°C) and protected from light.

Before use the identity, strength, purity and composition, or other characteristics which appropriately defined the batch from which the test material for this study was drawn, were determined by the Sponsor (Appendix 1). Stability of the test material and methods of synthesis, fabrication or derivation were documented by the Sponsor. At the request of the Sponsor, a sample of the test material (approximately 35 grams) was returned on completion of the treatment period for re-analysis to confirm the integrity and stability of the material, under the storage conditions at these laboratories. The sample was packed in dry ice (deep frozen). This requirement is not documented in the protocol. It is considered that this course of action did not compromise the integrity of the study as it would provide useful storage information. The certificate of analysis relating to this assay is presented in Appendix 1.

Before the consignment of the test material was used a 1 g representative sample was taken from the first aliquot of material used. This sample was placed in a well-closed glass container and stored in the archives under the conditions specified for the bulk supply of the test material

All dosages and concentrations are expressed in terms of the material received.

Formulation

The Enzyme preparation from *Aspergillus niger* (ARO-1) was prepared for administration as a solution in water obtained by reverse osmosis to provide the required dosages at a constant volume-dosage of 10 ml/ kg bodyweight. Control rats received the vehicle alone at the same volume-dosage.

Before any weighings took place the bulk container of the test material was inverted ten times. On each occasion that doses were formulated and for each group, the test material was pre-weighed into a suitable container. The vehicle (water obtained by reverse osmosis) was then added until the required amount of product was attained. The product was first hand-stirred and then magnetically-stirred for approximately one minute to ensure that the test material was fully dispersed in the water. The bulk preparation was subdivided into seven aliquots each of which were stored at 4°C. Each day one aliquot was issued to the unit for use on the following day. Once received in the animal unit the aliquot was retained at 4°C until required.

Quality control of dosage form

Detailed records of compound usage were maintained. The amount of test material necessary to prepare the formulations and the quantity actually used were determined on each occasion. The difference between these amounts was checked before the formulations were dispensed.

Information received from the Sponsor indicated that the test material was stable in water for 15 days when stored refrigerated (approximately 4°C). The formulations are stated by the Sponsor to form solutions and therefore homogeneity assessments were not included in this study.

A record of the weight of each formulation dispensed and the amount remaining after dosing was made. The balance was compared with the predicted usage as a check that the doses had been administered correctly. No significant discrepancy was found.

Samples of each formulation prepared for administration in Weeks 1 and 3 of treatment were deep frozen (approximately –20°C) and dispatched to the Sponsor for analysis. The results of these analyses are reported separately by the Sponsor.

Administration

Animals received the test material or vehicle control formulations by gavage at a volume-dosage of 10 ml/kg bodyweight. All animals were dosed in sequence of cage-number within each group, once each day, seven days per week. The volume administered to each animal was calculated from the most recently recorded bodyweight.

Duration of treatment

All animals were treated for at least four consecutive weeks and killed in the first two days of Week 5.

Treatment, and the recording of serial observations, continued for all surviving animals throughout the necropsy period.

SERIAL OBSERVATIONS

All observations described below were performed in cage number sequence, except where otherwise indicated.

Signs

Animals were inspected at least twice daily for evidence of reaction to treatment or ill-health. Any deviations from normal were recorded at the time in respect of nature and severity, date and time of onset, duration and progress of the observed condition, as appropriate.

Daily during the first week of treatment and twice weekly during Weeks 2 to 4 (middle and end of each week), detailed observations were recorded before and after dosing; these observations were recorded at the following times in relation to dosing:

Immediately before dosing.
Immediately after dosing on return of the animal to its cage.
On completion of dosing of each group.
Between one and two hours after completion of dosing of all groups.
As late as possible in the working day.

In addition a more detailed weekly examination, which included palpation, was performed on each animal.

Cages and cage-trays were inspected daily for evidence of animal ill-health, such as blood or loose faeces.

During the acclimatisation period, observations of the animals and their cages were recorded at least once per day.

Bodyweight

Each animal was weighed during the acclimatisation period, on the day that treatment commenced, at weekly intervals throughout the treatment period and before necropsy.

Food consumption

The weight of food supplied to each cage, that remaining and an estimate of any spilled was recorded for each week throughout the treatment period. From these records the mean weekly consumption per animal was calculated for each cage.

Food conversion efficiency

Group mean food conversion efficiencies were calculated for each week of the treatment period.

Ophthalmoscopy

Before commencement of treatment both eyes of all animals were examined by means of an indirect ophthalmoscope, after the instillation of 0.5% tropicamide. The structures examined included the following:

Adnexa
Conjunctiva
Cornea and sclera
Anterior chamber and iris (pupil dilated)
Lens and vitreous
Ocular fundus

During Week 4 of treatment, all animals from Groups 1 and 4 were similarly examined.

Haematology, peripheral blood

During Week 4 of treatment (before dosing) blood samples were obtained from all animals.

Blood samples were withdrawn from the retro-orbital sinus, with the animals held under Isoflurane anaesthesia, and collected into EDTA as anticoagulant. The animals were starved overnight before blood sample collection. Samples were taken and analysed in the Group order 1, 4, 2 and 3. All samples were examined for the following characteristics:

Using a Technicon H·1 haematology analyser -

Packed cell volume (Haematocrit; Hct)
Haemoglobin concentration (Hb)
Erythrocyte count (RBC)
Total and differential † leucocyte count (WBC)
Platelet count (Plt)
Mean cell haemoglobin concentration (MCHC)
Mean cell haemoglobin (MCH)
Mean cell volume (MCV)

† The equipment distinguishes neutrophils, lymphocytes, eosinophils, basophils, monocytes and a small proportion of large unstained cells (LUC). Large unstained cells are those that the H·1 haematology analyser is unable to clarify as belonging to any other classes.

Blood film - Romanowsky stain, examined by light microscopy for abnormal morphology and unusual cell types, including normoblasts.

Additional blood samples were taken into citrate anticoagulant and examined in respect of:

Prothrombin time (PT) - after Quick (1966), J. Clin. Pathol. 45, 105.

Activated partial thromboplastin time (APTT) - after Proctor and Rapaport (1972), Am. J. Clin. Pathol. 36, 212.

Analysis of these samples revealed a large number of the samples taken into citrate (both sexes) were clotted and an unacceptable number of samples taken into EDTA were clotted in the Group 3 females. A repeat sampling occasion was scheduled (Week 5). Animals were not starved prior to this bleed. The males were sampled in the unit prior to their dispatch to necropsy; females were sampled in necropsy and were not allowed to recover from the anaesthesia prior to their sacrifice. The data from these repeat samples are presented in the report. All data from the first unsuccessful bleed are not presented but are retained in the archives.

Blood chemistry

At the same time as samples were taken for peripheral haematology in Week 4, further blood samples were taken from all animals and collected into lithium heparin as anticoagulant. Samples were taken and analysed in the same sequence as for peripheral haematology. After separation the plasma was examined in respect of:

Alkaline phosphatase activity (Alk. Phos) - after Tietz *et al.* (1980), Clin Chem. 26(7), 1023.

Alanine amino-transferase activity (ALT) - by the method defined by the International Federation of Clinical Chemistry, Committee on Standards, Enzyme Panel (1978), Clin. Chem. 24, 720-721.

Aspartate amino-transferase activity (AST) - by the method defined by the International Federation of Clinical Chemistry, Committee on Standards, Enzyme Panel (1978), Clin. Chem. 24, 720-721.

Gamma-glutamyl transpeptidase activity (gGT) - after Szasz *et al.* (1969), Clin. Chem. 15, 124.

Ornithine carbamyl transferase activity (OCT) - after Ceriotti (1973), Clin. Chim. Acta. 47, 97.

Glucose concentration (Gluc) - after Bondor and Mead (1974), Clin. Chem. 20, 586.

Total bilirubin concentration (Bili.Total) - after Walters and Gerarde (1970), Microchem. J. 15, 231.

Total cholesterol concentration (Chol Total) - after Siedel *et al.* (1981), J. Clin. Chem. Clin. Biochem. 19, 838.

Total triglyceride concentration (Trig) - after Fossati and Prencipe (1982), Clin. Chem., 28, 2077.

Urea concentration (Urea) - after Talke and Schubert (1965), Klin. Wochenschr. 43, 174.

Creatinine concentration (Creat) - after Henry (1974), in "Clin. Chem. Principles and Technics", 2nd Edition, Harper and Row, Hagerstown Md.

Total protein concentration (Total Prot) - after Weichselbaum (1946), Am. J. Clin. Pathol. Tech. Sect. 10, 40-49.

Albumin concentration (Alb) - after Doumas *et al.* (1971), Clin. Chem. Acta. 31, 87.

Albumin/globulin ratio (A/G ratio) - calculated from total protein concentration and chemically analysed albumin concentration.

Sodium (Na) and potassium (K) - by indirect ion-selective electrode on the Technicon AXON.

Chloride (Cl) - after Schoenfeld and Lewellen (1964), Clin. Chem. 10, 533.

Calcium concentration (Ca) - after Young *et al.* (1975), Clin. Chem. 21, No. 5.

Inorganic phosphorus (Phos) - after Daly and Ertingshausen (1972), Clin. Chem., 18, 263-265.

TERMINAL OBSERVATIONS

Euthanasia

Animals were killed by carbon dioxide inhalation at the end of the scheduled treatment period.

The sequence in which the animals were killed after completion of treatment was selected to allow satisfactory inter-group comparison.

Macroscopic pathology

All animals were subjected to a detailed necropsy.

The necropsy procedure included a review of the history of each animal and a detailed examination of the external features and orifices, the neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera. The requisite organs were weighed and external and cut surfaces of the organs and tissues were examined as appropriate. Abnormalities and interactions were noted and the required tissue samples preserved in fixative.

Before disposal of the carcase the retained tissues were checked against the protocol and a senior prosector reviewed the necropsy report.

Organ weights

The following organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy.

Brain
Epididymides
Heart
Kidneys
Liver
Lungs with mainstem bronchi
Ovaries
Spleen
Testes
Thymus
Thyroid with parathyroids, after partial fixation
Uterus with cervix

Tissues preserved for histopathology

Samples of the following tissues were preserved in 10% neutral buffered formalin, except eyes which were placed in Davidson's fluid and testes and epididymides which were placed in Bouin's fluid and subsequently retained in 70% industrial methylated spirit.

Adrenals
Aorta – thoracic
Brain
Caecum
Colon
Duodenum
Epididymides
Eyes
Femoral bone
Heart
Ileum
Jejunum
Kidneys
Lachrymal glands
Liver
Lungs with mainstem bronchi
Lymph nodes - mandibular
- mesenteric
Mammary area – caudal
Oesophagus
Ovaries
Pancreas
Pituitary
Prostate
Rectum
Salivary gland
Sciatic nerve, one only
Seminal vesicles
Skeletal muscle - thigh, one only
Spinal cord
Spleen
Sternum
Stomach
Testes
Thymus
Thyroid with parathyroids
Tongue
Trachea
Urinary bladder
Uterus with cervix
Vagina

Samples of any abnormal tissues were also retained for histopathological examination. In those cases where a lesion was not clearly delineated, contiguous tissue was fixed with the grossly affected region and sectioned as appropriate.

Tissues preserved, but not examined

Samples of the tissues listed below were not processed histologically, but are held in fixative against any future requirement for microscopic examination.

Head - including nasal cavity, paranasal sinuses and nasopharynx.
Sciatic nerve, one only.
Skeletal muscle - thigh, one only.

Histology

Tissue samples from all animals specified below were dehydrated, embedded in paraffin wax, sectioned at approximately four to five micron thickness and stained with haematoxylin and eosin.

Tissue		Regions to be examined
Adrenals	:	cortex and medulla.
Brain	:	cerebellum, cerebrum and midbrain.
Femur	:	longitudinal section through joint, to include articular surface, epiphysial plate and bone marrow.
Heart	:	including auricular and ventricular regions.
Kidneys	:	including cortex, medulla and papilla regions.
Liver	:	section from all main lobes.
Lungs	:	section from two major lobes, to include bronchi.
Mammary area	:	including overlying skin.
Spinal cord	:	transverse and longitudinal sections at the cervical level.
Sternum	:	including bone marrow.
Stomach	:	keratinised, glandular and antrum.
Thyroid	:	including parathyroid in section, where possible.
Uterus	:	uterus section separate from cervix section.

For bilateral organs sections of both left and right organs were examined. A single section was prepared from each of the remaining tissues required for microscopic pathology.

Microscopy

Microscopic examination was performed as follows:

i. The tissues specified above were examined for all animals of Groups 1 and 4.

ii The caecums, which were considered to exhibit a reaction to treatment at the high dosage in the females, were examined for all females of Groups 2 and 3.

iii. Tissues reported at macroscopic examination as being abnormal were examined for all animals.

Findings were either reported as "Present" or assigned a severity grade. In the latter case one of the following five grades was used - minimal, slight, moderate, marked or severe.

DEFINITION OF "WEEK"

The first week of treatment started at midnight prior to treatment commencing and ended at midnight on the seventh day following. Subsequent experimental weeks were of the same duration. Bodyweights taken on the first day of treatment, prior to the animals being dosed, are designated as Day 0.

TREATMENT OF DATA

This report contains serial observations pertaining to all weeks of treatment completed before commencement of the necropsies, together with signs data collected during the necropsy period. The only serial observation relating to the acclimatisation period included in this report relate to ophthalmoscopy.

Group mean values were calculated from the individual values presented in the appendices, unless otherwise specified below.

The death code in the appendices has the following meaning:

7 Terminal kill.

Throughout the tables the following abbreviations are used:

N Number of animals examined.
SD Standard deviation.

In all text and word processed documents the test material is referred to as *Aspergillus niger* (ARO-1). Due to limitations of the computer data collection system, the headings on the prints included in this report do not have the test material name italicised.

Signs

Individual incidences in Appendix 3 are presented as the weeks in which signs were observed. The number of animals affected was summed for the group and presented in Table 1.

The only sign evident at dosing was observed in one female receiving 1500mg/kg/day (No. 68) which on Day 5 of the treatment period showed body tremors immediately after dosing and on completion of dosing the group. Consequently, as no other signs were recorded during the study, no "dosing signs" data are presented in this report.

Bodyweight

Group mean weight changes were calculated from the weight changes of individual animals.

Food consumption

Weekly group mean food consumptions and standard deviations were derived from unrounded cage values.

Overall food consumption values were calculated from the weekly group mean values presented.

Food conversion efficiency

The weekly group mean values presented were calculated by first deriving the weekly cage values. These were calculated from the bodyweight gain of animals at the end of the week and the total weight of food consumed in the cage. Weekly group means were derived from unrounded cage values.

Overall group mean values were calculated from the total weight gain (Week 0 to 4) divided by the total food intake (Weeks 1 to 4) and multiplied by 100 to give a percentage.

Ophthalmoscopy

Whilst all observations made at ophthalmoscopic examination are recorded in the raw data, this report only contains those that were considered to be unusual or abnormal. Observations were bilateral unless otherwise indicated.

Haematology

Differential leucocyte counts were determined automatically by counting the numbers of lymphocytes, neutrophils, monocytes, eosinophils, basophils and large unstained cells in the instrument sample.

The units for erythrocyte count, total and differential leucocyte count and platelet count represent the number of cells per litre of blood; for example x10-12/L indicates 10^{12}/l.

Blood Chemistry

Albumin to globulin (A/G) ratios were calculated as:

$$A/G = \frac{\text{Chemical Albumin concentration}}{\text{Total Protein} - \text{Chemical Albumin concentration}}$$

Pathology

Tissues which could not be examined are specified in the appendix. The absence of a comment for a tissue scheduled for examination therefore indicates that the tissue was examined and found to be normal. In all tabular presentations of data the tissues specified in the protocol for histopathological examination precede other tissues.

Statistical analysis

The significance of inter-group differences in haematology (excluding the incidence of morphological abnormalities evident on blood smears) and blood chemistry was assessed by Student's t-test using a pooled error variance. Statistical significances for eosinophil, basophil, monocyte and large unstained cell counts are not reported as these data are not considered to be normally distributed.

For organ weights and bodyweight changes, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used.

Inter-group differences in macroscopic pathology and histopathology were assessed using Fisher's Exact test.

Unless stated, group mean values or incidences for the treated groups were not significantly different from those of the Controls ($p>0.05$).

RESULTS

Signs and mortality (Table 1; Appendix 2)

There were no deaths. There were no signs seen on this study which were considered to be related to treatment with Enzyme preparation from *Aspergillus niger* (ARO-1) .

Bodyweight (Figures 2A and B; Table 2; Appendix 3)

Bodyweights were considered to have been unaffected by treatment.

The weight gains of females receiving the Enzyme preparation from *Aspergillus Niger* (ARO-1) were lower than those of the Controls. The differences were, however, slight and were not dosage related. Consequently, they were considered to represent normal biological variation.

Food consumption (Table 3; Appendix 4)

Food intake was unaffected by treatment.

Treated females consumed slightly less food than the Controls, but the difference was small and not dosage-related. Consequently, these variations were considered to be unrelated to treatment.

Food conversion efficiency (Table 4)

Food conversion efficiencies were not affected by treatment.

Ophthalmoscopy (Appendix 5)

There were no ophthalmic findings considered to be related to treatment with Enzyme preparation from *Aspergillus niger* (ARO-1).

Haematology (Table 5; Appendix 6)

There were no haematological changes that were attributed to treatment.

Inter-group variations occasionally achieved statistical significance ($p<0.05$) but they were slight and not dosage related and were, therefore, not considered to be related to treatment. Such changes included the slight increase in platelet counts in females receiving 5000 mg/kg/day which was attributed to slightly high values in two animals. Consequently, this difference from Controls was considered fortuitous.

Blood chemistry (Table 6; Appendix 7)

Slightly low plasma phosphorus concentrations, compared with the Controls, were observed in animals receiving 5000 mg/kg/day and in males receiving 1500 mg/kg/day.

Other inter-group differences from Controls, although occasionally attaining statistical significance ($p<0.05$), lacked dosage-relationship and were not attributable to treatment. Such changes included marginally low plasma calcium concentrations in all treated male groups; this was not dosage-related. Evaluation of the background Control data indicates that the Control values were unusually high (mean value of 2.60 mmol/l) and that the values seen in the treated groups (2.45, 2.43 and 2.53 mmol/l) are more representative for rats of this strain and age at these laboratories. In a sample of 313 male rats of a similar age, the mean value for plasma calcium was 2.50 mmol/l with a standard deviation of 0.128 giving a normal range (± 2 standard deviations) for this parameter of 2.24 to 2.76 mmol/l. The variations in plasma calcium concentration were, therefore, considered fortuitous.

Organ weights (Table 7; Appendix 8A-B)

Analysis of the absolute and bodyweight relative organ weights did not reveal any effect of treatment.

Marginally low uterus weights were recorded for females which had received 5000 mg/kg/day; statistical significance was not attained. No macropathological changes were seen in these tissues and this slight change in organ weight was considered to be a chance occurrence and of no toxicological importance.

Macroscopic pathology (Table 8; Appendix 9)

There were no macroscopic findings seen at necropsy which were considered to be related to treatment. Findings seen in the animals were of the kind commonly seen in rats of this age and strain at these laboratories.

Microscopic pathology (Table 9; Appendix 9)

When compared with the Controls, a higher incidence of inflammatory cell infiltrate in the lamina propria of the caecum was evident in treated females. The severity of this finding was graded as slight in all animals affected. This finding was recorded in three females which had received 500 mg/kg/day, in two which had received 1500 mg/kg/day and in seven which had received 5000 mg/kg/day.

All other microscopic findings were considered to be incidental and of no toxicological importance.

DISCUSSION

Treatment of CD rats with Enzyme preparation from *Aspergillus niger* (ARO-1) at dosages up to 5000 mg/kg/day for four weeks was well-tolerated, producing no significant effects.

The slightly low plasma phosphorus concentrations at 5000 mg/kg/day in both sexes and in males receiving 1500 mg/kg/day was not dosage-related in the males and there were no histopathological findings that would account for this finding. Small changes in phosphate levels do not appear critical to health and, as an isolated blood chemistry finding, this change is not considered to be of toxicological significance.

Microscopic evaluation of the tissues revealed one finding associated with treatment. When compared with the Controls, a higher incidence of inflammatory cell infiltrate in the lamina propria of the caecum was seen in treated females. The presence of the test material in the alimentary tract may have caused minor irritation in the caecum. This finding is rodent-specific and is not considered to be a toxic effect of treatment.

CONCLUSION

Treatment of CD rats with Enzyme preparation from *Aspergillus niger* (ARO-1) at dosages of 500, 1500 and 5000 mg/kg/day resulted in some minor changes. All changes were considered to be of no toxicological significance. The dosage of 5000 mg/kg/day is considered to be the No-Observed-Adverse-Effect-Level (NOAEL) in this study.

FIGURE 1

Cage arrangement in batteries

Group	:	1	2	3	4
Compound	:	Control	-------*Aspergillus niger* (ARO-1)-------		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group/sex
Cage number
Animal numbers

Battery 1 - males

2M 3 11-15	1M 1 1-5	3M 5 21-25
4M 7 31-35	2M 4 16-20	1M 2 6-10
3M 6 26-30	4M 8 36-40	

Battery 2 - females

2F 11 51-55	1F 9 41-45	3F 13 61-65
4F 15 71-75	2F 12 56-60	1F 10 46-50
3F 14 66-70	4F 16 76-80	

FIGURE 2A

Group mean bodyweight versus period of treatment - Males.

Group	:	1	2	3	4
Compound	:	Control	------*Aspergillus niger (ARO-1)*------		
Dosage (mg/kg/day)	:	0	500	1500	5000

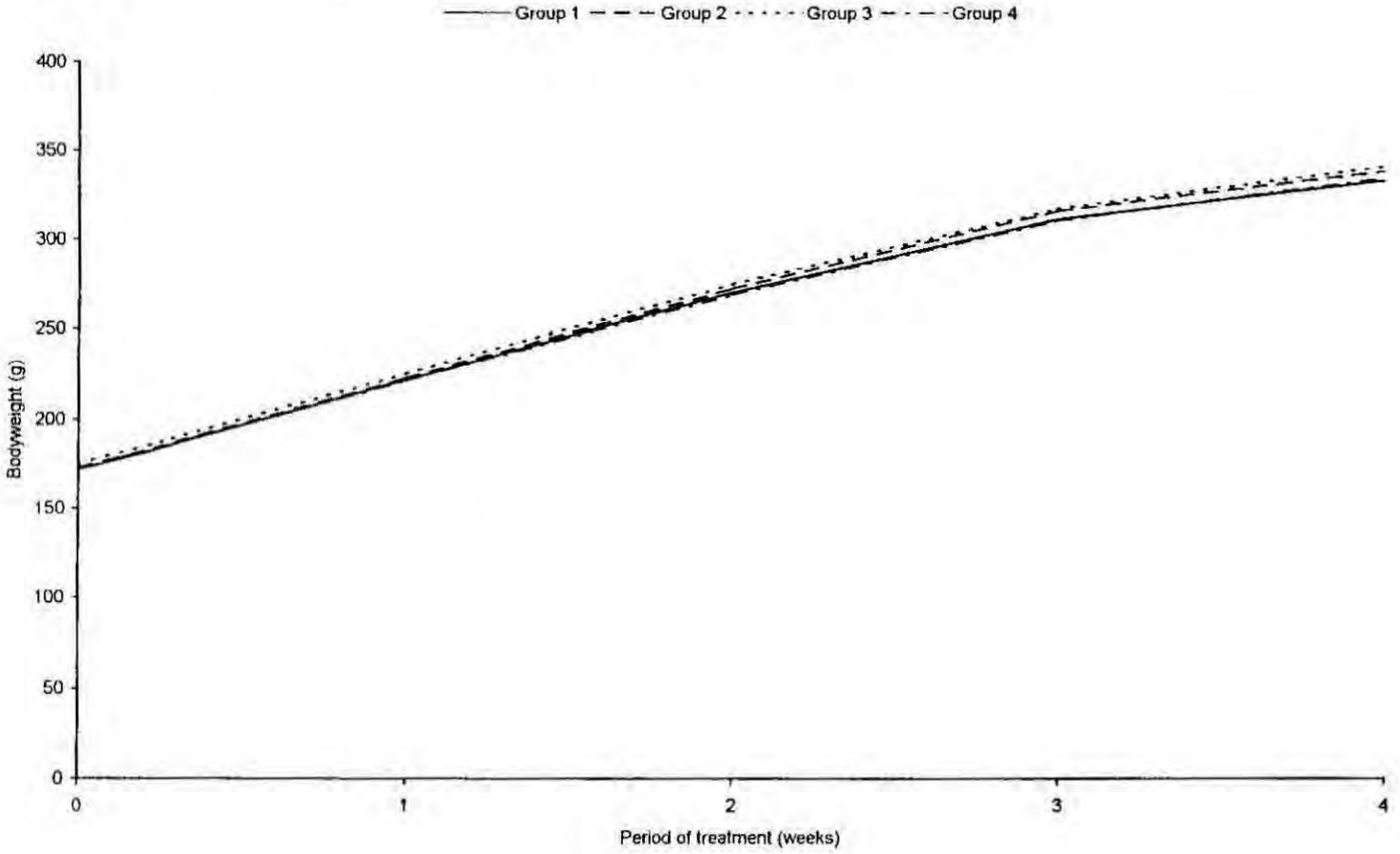

FIGURE 2B

Group mean bodyweight versus period of treatment - Females.

Group	:	1	2	3	4
Compound	:	Control	-------*Aspergillus niger (ARO-1)*-------		
Dosage (mg/kg/day)	:	0	500	1500	5000

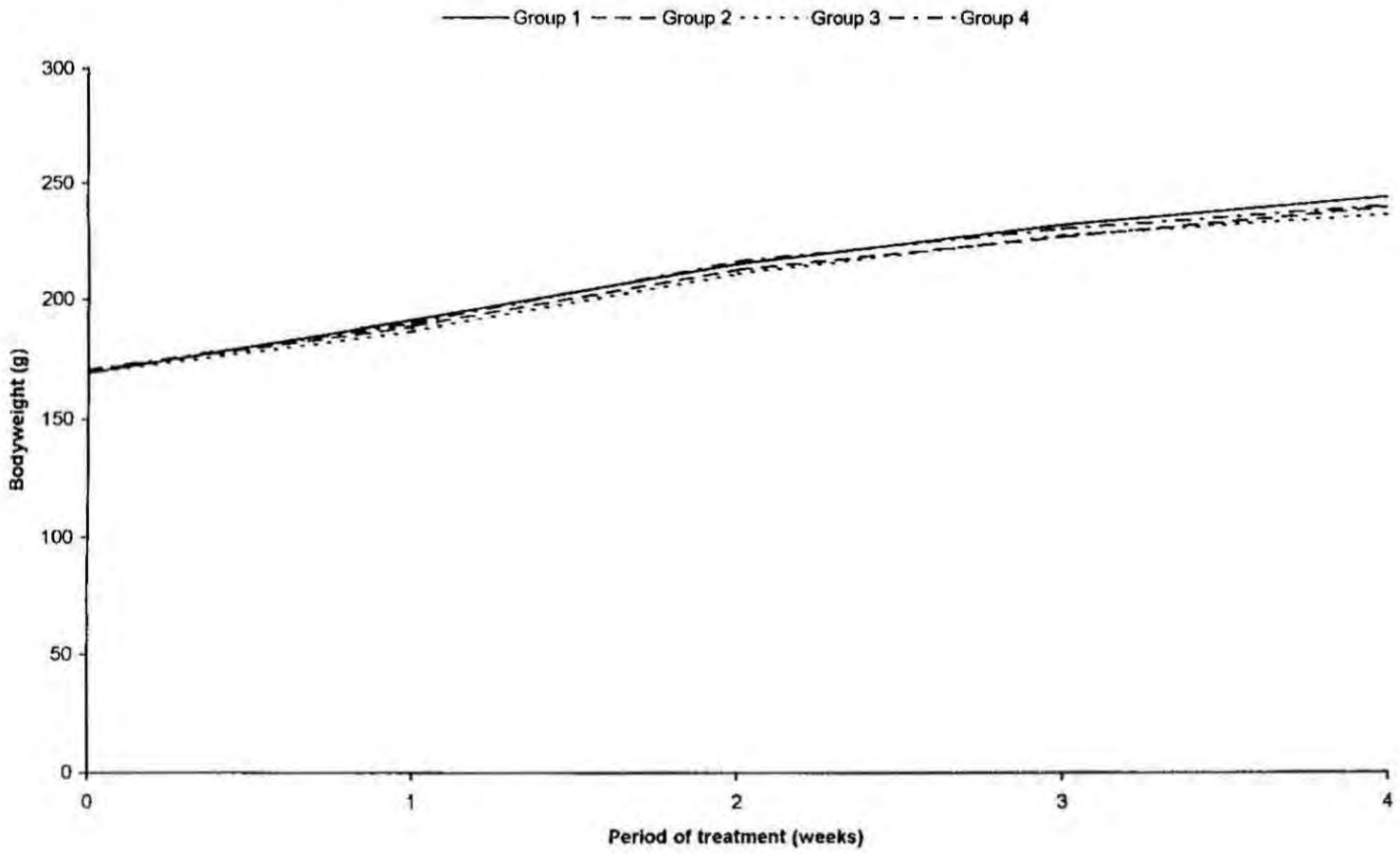

TABLE 1

Print No: 0001

Signs - group distribution of observations

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 061

WEEKS 1-5

NUMBER OF ANIMALS AFFECTED

CATEGORY / KEYWORD / QUALIFIER	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	NUMBER:	10	10	10	10	10	10	10	10
*** TOP OF LIST ***									
BUILD (DEFORMITY)									
PARTIALLY ABSENT APPENDAGE									
RIGHT PINNA		0	0	0	0	0	0	1	0
BEHAVIOUR									
VOCALIZATION		0	0	0	0	1	0	0	0
COAT									
HAIRLOSS									
FORELIMBS		0	0	0	0	0	0	1	0
HEAD		0	0	0	0	0	0	2	0
DORSAL BODY SURFACE		3	0	0	0	0	0	0	0
EYES									
LARGE									
RIGHT		0	0	0	0	0	1	0	0
*** END OF LIST ***									

TABLE 2

Print No: 0002

Bodyweight - group mean values (g)

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 061

	SEX:	MALE				FEMALE			
WEEK	GROUP:	1	2	3	4	1	2	3	4
0	N	10	10	10	10	10	10	10	10
	MEAN	172	173	175	173	169	170	169	171
	S.D.	14.7	11.7	11.2	10.4	8.4	8.3	6.4	11.7
1	N	10	10	10	10	10	10	10	10
	MEAN	222	223	226	222	192	189	187	190
	S.D.	19.8	14.8	13.6	14.8	13.6	9.5	9.9	13.9
2	N	10	10	10	10	10	10	10	10
	MEAN	270	272	275	269	215	213	211	216
	S.D.	22.5	17.7	17.4	22.7	17.5	10.8	12.8	12.6
3	N	10	10	10	10	10	10	10	10
	MEAN	311	316	317	310	232	227	227	230
	S.D.	24.7	22.1	19.2	29.9	18.3	12.5	15.1	12.7
4	N	10	10	10	10	10	10	10	10
	MEAN	332	338	340	333	244	240	236	240
	S.D.	29.1	24.6	19.3	35.5	19.7	16.8	16.5	11.6
Gain Week 0 to 4		161	165	165	161	74	69	67	69
As % of Control		-	102	102	100	-	93	91	93

TABLE 3

Print No: 0003

Food consumption - group mean values (g/animal)

Printed: 10-JAN-00
Page: 1

Schedule number: GSB 061

Group	:	1	2	3	4
Compound	:	Control	Aspergillus niger (ARO-1)		
Dosage (mg/kg/day)	:	0	500	1500	5000

	SEX:	MALE				FEMALE			
WEEK	GROUP:	1	2	3	4	1	2	3	4
1	N	10	10	10	10	10	10	10	10
	MEAN	189	185	192	190	141	135	136	135
	S.D.	6.5	6.1	3.5	6.2	0.8	6.3	2.3	6.7
2	N	10	10	10	10	10	10	10	10
	MEAN	196	194	199	198	145	137	141	140
	S.D.	1.7	6.5	0.3	7.5	0.1	5.7	4.0	5.3
3	N	10	10	10	10	10	10	10	10
	MEAN	192	195	205	205	152	138	143	141
	S.D.	1.0	1.1	1.6	9.0	1.8	1.9	9.1	6.7
4	N	10	10	10	10	10	10	10	10
	MEAN	179	176	185	182	148	137	142	142
	S.D.	3.1	4.6	7.0	10.8	1.2	6.4	7.1	6.5
Total Weeks 1-4		756	750	781	775	586	547	562	558
As % of Control		-	99	103	103	-	93	96	95

TABLE 4

Print No: 0004

Food conversion efficiency - group mean values (%)

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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Page: 1

Schedule number: GSB 061

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
WEEK								
1	26.7	27.4	26.3	25.9	15.8	13.8	13.1	14.3
2	24.4	25.3	24.6	23.7	16.1	17.2	16.9	18.7
3	21.3	22.3	20.5	20.2	11.1	10.2	11.3	9.8
4	11.9	12.4	12.6	12.6	8.1	9.2	6.2	7.0
Overall Weeks 1-4	21.3	22.0	21.1	20.8	12.6	12.6	11.9	12.4

TABLE 5

Haematology – group mean values during Week 4/5 of treatment

Group	:	1	2	3	4
Compound	:	Control	-------*Aspergillus niger* (ARO-1)-------		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Hct	Hb	RBC	MCH	MCHC	MCV	WBC	Neutrophil	Lymphocyte
		L/L	g/dL	x10-12/L	pg	g/dL	fL	x10-9/L	x10-9/L	x10-9/L
1M										
	Mean	0.444	15.0	7.49	20.0	33.7	59.3	12.02	1.53	9.82
	SD	0.0176	0.59	0.208	0.68	0.33	1.97	1.769	0.502	1.428
	n	9	9	9	9	9	9	9	9	9
2M										
	Mean	0.433	14.4 [a]	7.33	19.7	33.3	59.1	11.60	1.37	9.53
	SD	0.0156	0.55	0.203	0.61	0.32	1.85	4.297	0.820	3.091
	n	9	9	9	9	9	9	9	9	9
3M										
	Mean	0.432	14.5	7.27	19.9	33.5	59.4	12.05	1.28	10.12
	SD	0.0158	0.49	0.350	0.49	0.47	1.41	2.316	0.409	1.974
	n	10	10	10	10	10	10	10	10	10
4M										
	Mean	0.443	14.8	7.44	19.9	33.3	59.6	10.71	1.42	8.65
	SD	0.0220	0.61	0.455	0.60	0.58	1.90	2.274	0.547	2.090
	n	10	10	10	10	10	10	10	10	10

Significant when compared with Group 1: a - $p<0.05$.

TABLE 5 - continued

Haematology - group mean values during Week 4/5 of treatment

Group	:	1	2	3	4
Compound	:	Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Eosinophil	Basophil	Monocyte	LUC	Plt	PT	APTT
		x10-9/L	x10-9/L	x10-9/L	x10-9/L	x10-9/L	sec	sec
1M								
	Mean	0.12	0.04	0.28	0.23	1056	12.3	17.1
	SD	0.050	0.013	0.097	0.075	132.1	0.56	2.75
	n	9	9	9	9	9	10	10
2M								
	Mean	0.08	0.04	0.36	0.21	1082	12.0	14.4
	SD	0.033	0.033	0.251	0.108	227.3	0.61	3.53
	n	9	9	9	9	9	10	7
3M								
	Mean	0.09	0.04	0.31	0.20	1107	11.9	14.9
	SD	0.046	0.017	0.169	0.065	154.0	0.44	2.41
	n	10	10	10	10	10	9	8
4M								
	Mean	0.08	0.03	0.33	0.20	1107	12.2	16.0
	SD	0.018	0.013	0.106	0.071	153.1	0.37	3.13
	n	10	10	10	10	10	9	9

TABLE 5 - continued

Haematology - group mean values during Week 4/5 of treatment

Group	: 1	2	3	4
Compound	: Control	—— *Aspergillus niger* (ARO-1) ——		
Dosage (mg/kg/day)	: 0	500	1500	5000

Group		Hct	Hb	RBC	MCH	MCHC	MCV	WBC	Neutrophil	Lymphocyte
		L/L	g/dL	x10-12/L	pg	g/dL	fL	x10-9/L	x10-9/L	x10-9/L
1F										
	Mean	0.383	13.0	6.42	20.2	33.9	59.6	8.59	1.49	6.56
	SD	0.0130	0.52	0.256	0.63	0.71	1.21	1.766	0.795	1.854
	n	9	9	9	9	9	9	9	9	9
2F										
	Mean	0.389	13.1	6.54	20.0	33.7	59.5	10.29	1.71	8.01
	SD	0.0135	0.39	0.300	0.60	0.53	1.60	3.851	0.629	3.581
	n	10	10	10	10	10	10	10	10	10
3F										
	Mean	0.385	13.1	6.62	19.8	34.0	58.2	12.47 [b]	1.77	9.86 [b]
	SD	0.0134	0.46	0.255	0.75	0.49	1.85	2.413	0.728	2.015
	n	8	8	8	8	8	8	8	8	8
4F										
	Mean	0.381	12.9	6.54	19.8	33.9	58.4	9.83	1.43	7.94
	SD	0.0116	0.45	0.365	0.57	0.36	1.95	2.092	0.505	1.518
	n	10	10	10	10	10	10	10	10	10

Significant when compared with Group 1: b - $p<0.01$.

TABLE 5 - continued

Haematology - group mean values during Week 4/5 of treatment

Group	:	1	2	3	4
Compound	:	Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Eosinophil	Basophil	Monocyte	LUC	Plt	PT	APTT
		x10-9/L	x10-9/L	x10-9/L	x10-9/L	x10-9/L	sec	sec
1F								
	Mean	0.14	0.02	0.23	0.15	1113	13.4	16.6
	SD	0.033	0.009	0.063	0.040	96.3	0.38	1.59
	n	9	9	9	9	9	10	10
2F								
	Mean	0.12	0.03	0.25	0.16	1156	13.7	14.0 [a]
	SD	0.037	0.019	0.095	0.059	111.9	0.32	2.27
	n	10	10	10	10	10	8	8
3F								
	Mean	0.21	0.04	0.38	0.21	1068	14.2 [a]	15.3
	SD	0.081	0.015	0.141	0.073	97.9	0.98	2.60
	n	8	8	8	8	8	10	8
4F								
	Mean	0.13	0.02	0.17	0.13	1241 [a]	13.8	16.3
	SD	0.056	0.018	0.064	0.045	136.8	0.66	1.49
	n	10	10	10	10	10	10	10

Significant when compared with Group 1: a - $p<0.05$.

TABLE 6

Blood Chemistry - group mean values during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Alk. Phos	ALT	AST	gGT	OCT	Bili. Total	Urea	Creat	Gluc
		U/L	U/L	U/L	U/L	U/L	umol/L	mmol/L	umol/L	mmol/L
1M										
	Mean	204	40	88	0	5.4	0	4.28	44	5.30
	SD	16.5	5.6	6.8	0.5	0.82	0.0	0.608	2.8	0.543
	n	10	10	10	10	10	10	10	10	10
2M										
	Mean	225	43	84	1	5.3	0	4.47	43	5.37
	SD	39.5	7.4	10.2	0.6	0.70	0.3	0.535	3.0	0.486
	n	10	10	10	10	10	10	10	10	10
3M										
	Mean	204	41	86	1	5.8	0	4.44	43	5.71
	SD	26.9	8.1	9.9	0.7	0.69	0.3	0.700	2.9	0.749
	n	10	10	10	10	10	10	10	10	10
4M										
	Mean	214	41	84	1	5.3	0	4.68	44	5.79
	SD	35.5	6.1	9.9	0.5	1.28	0.0	0.420	2.3	0.713
	n	10	10	10	10	10	10	10	10	10

TABLE 6 - continued

Blood Chemistry - group mean values during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	-------*Aspergillus niger* (ARO-1)-------		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Chol Total	Trig	Na	K	Cl	Ca Total	Phos	Total Prot	Alb
		mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	g/L	g/L
1M										
	Mean	2.20	0.76	142	3.7	105	2.60	2.87	60	39
	SD	0.293	0.134	1.1	0.20	1.4	0.055	0.161	1.8	1.2
	n	10	10	10	10	10	10	10	10	10
2M										
	Mean	2.15	0.98	142	4.1 ^{a}	107 ^{b}	2.45 ^{c}	2.77	58 ^{a}	38
	SD	0.252	0.325	1.2	0.41	1.2	0.047	0.129	1.3	0.9
	n	10	10	10	10	10	10	10	10	10
3M										
	Mean	2.27	0.87	142	4.0	106 ^{a}	2.43 ^{c}	2.66 ^{b}	58 ^{a}	38
	SD	0.420	0.250	1.5	0.19	2.2	0.062	0.112	2.3	1.4
	n	10	10	10	10	10	10	10	10	10
4M										
	Mean	2.06	0.84	142	3.9	105	2.53 ^{b}	2.73 ^{a}	59	38
	SD	0.244	0.213	1.4	0.22	1.5	0.052	0.169	2.3	1.8
	n	10	10	10	10	10	10	10	10	10

Significant when compared with Group 1: a - $p<0.05$; b - $p<0.01$; c - $p<0.001$.

TABLE 6 - continued

Blood Chemistry - group mean values during Week 4 of treatment

Group	: 1	2	3	4
Compound	: Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	: 0	500	1500	5000

Group		A/G Ratio
1M		
	Mean	1.83
	SD	0.186
	n	10
2M		
	Mean	1.92
	SD	0.117
	n	10
3M		
	Mean	1.87
	SD	0.042
	n	10
4M		
	Mean	1.74
	SD	0.143
	n	10

TABLE 6 - continued

Blood Chemistry - group mean values during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Alk. Phos	ALT	AST	gGT	OCT	Bili. Total	Urea	Creat	Gluc
		U/L	U/L	U/L	U/L	U/L	umol/L	mmol/L	umol/L	mmol/L
1F										
	Mean	153	33	86	0	6.1	0	5.61	50	5.39
	SD	25.1	5.0	8.7	0.4	1.18	0.0	0.730	2.9	0.461
	n	10	10	10	10	10	10	10	10	10
2F										
	Mean	124[a]	34	85	0	6.5	0	6.60[a]	50	6.05[a]
	SD	23.1	5.8	8.1	0.3	1.65	0.0	1.002	4.9	0.668
	n	10	10	10	10	10	10	10	10	10
3F										
	Mean	143	37	92	0	5.5	0	5.82	49	5.98[a]
	SD	29.7	14.0	19.4	0.4	0.65	0.0	0.617	2.8	0.558
	n	10	10	10	10	10	10	10	10	10
4F										
	Mean	129	38	89	0	6.0	0	5.18	49	5.61
	SD	27.9	6.7	11.1	0.4	1.20	0.0	0.948	2.2	0.528
	n	10	10	10	10	10	10	10	10	10

Significant when compared with Group 1: a - $p<0.05$.

TABLE 6 - continued

Blood Chemistry - group mean values during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	:	0	500	1500	5000

Group		Chol Total	Trig	Na	K	Cl	Ca Total	Phos	Total Prot	Alb
		mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	g/L	g/L
1F										
	Mean	2.32	0.41	141	3.3	107	2.53	2.30	62	42
	SD	0.406	0.101	0.9	0.15	0.8	0.056	0.171	1.6	1.8
	n	10	10	10	10	10	10	10	10	10
2F										
	Mean	2.41	0.50	142 [a]	3.6 [b]	107	2.54	2.20	62	43
	SD	0.350	0.073	1.2	0.25	1.3	0.058	0.210	2.6	1.6
	n	10	10	10	10	10	10	10	10	10
3F										
	Mean	2.34	0.56 [b]	142	3.6 [b]	107	2.53	2.26	62	43 [a]
	SD	0.288	0.136	1.6	0.32	1.6	0.054	0.095	2.1	2.5
	n	10	10	10	10	10	10	10	10	10
4F										
	Mean	2.33	0.49	142	3.5	108 [b]	2.53	2.06 [b]	63	43
	SD	0.394	0.069	1.3	0.18	1.1	0.033	0.191	1.8	1.3
	n	10	10	10	10	10	10	10	10	10

Significant when compared with Group 1: a - $p<0.05$; b - $p<0.01$.

TABLE 6 - continued

Blood Chemistry - group mean values during Week 4 of treatment

Group	: 1	2	3	4
Compound	: Control	----- *Aspergillus niger* (ARO-1) -----		
Dosage (mg/kg/day)	: 0	500	1500	5000

Group		A/G Ratio
1F		
	Mean	2.06
	SD	0.221
	n	10
2F		
	Mean	2.27 [a]
	SD	0.133
	n	10
3F		
	Mean	2.29 [b]
	SD	0.221
	n	10
4F		
	Mean	2.07
	SD	0.128
	n	10

Significant when compared with Group 1: a - $p<0.05$; b - $p<0.01$.

TABLE 7A

Print No: 0008

Absolute organ weights - group mean values (g) for animals killed after 4 weeks of treatment

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
TERMINAL BODY WEIGHT (g)								
		DUNNETT'S TEST				DUNNETT'S TEST		
N :	10	10	10	10	10	10	10	10
MEAN :	324.1	331.6	331.3	324.1	241.9	236.2	238.5	240.1
sd :	27.6	24.7	18.7	35.1	20.1	14.9	16.9	11.4
BRAIN								
		DUNNETT'S TEST				DUNNETT'S TEST		
N :	10	10	10	10	10	10	10	10
MEAN :	1.96	1.98	1.96	1.96	1.93	1.97	1.91	1.90
sd :	0.08	0.09	0.07	0.10	0.09	0.07	0.06	0.07
EPIDIDYMIDES								
		DUNNETT'S TEST						
N :	10	10	10	10				
MEAN :	0.771	0.800	0.826	0.780				
sd :	0.089	0.055	0.061	0.067				
HEART								
		DUNNETT'S TEST				DUNNETT'S TEST		
N :	10	10	10	10	10	10	10	10
MEAN :	1.26	1.20	1.26	1.22	0.98	0.99	0.95	0.96
sd :	0.09	0.12	0.09	0.07	0.11	0.12	0.11	0.10
KIDNEYS								
		DUNNETT'S TEST				DUNNETT'S TEST		
N :	10	10	10	10	10	10	10	10
MEAN :	2.42	2.54	2.42	2.37	1.94	1.80	1.93	1.88
sd :	0.24	0.28	0.21	0.26	0.19	0.14	0.12	0.18

TABLE 7A - continued.

Print No: 0008

Absolute organ weights - group mean values (g) for animals killed after 4 weeks of treatment

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 061

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
LIVER								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	13.6	14.5	14.3	13.3	10.7	9.7	9.5 a	10.3
sd :	1.8	2.3	1.3	1.8	1.2	1.1	0.9	0.7
LUNGS & BRONCHI								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	1.42	1.43	1.50	1.49	1.22	1.22	1.23	1.22
sd :	0.18	0.13	0.12	0.16	0.11	0.16	0.08	0.07
OVARIES								
					DUNNETT'S TEST			
N :					10	10	10	10
MEAN :					0.087	0.080	0.082	0.086
sd :					0.012	0.012	0.009	0.012
SPLEEN								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.695	0.702	0.804	0.727	0.565	0.516	0.516	0.529
sd :	0.133	0.100	0.056	0.137	0.119	0.102	0.084	0.071
TESTES								
	DUNNETT'S TEST							
N :	10	10	10	10				
MEAN :	3.19	3.23	3.39	3.18				
sd :	0.31	0.14	0.25	0.27				

Significant when compared with Group 1: a - $p<0.05$

TABLE 7A - continued. Print No: 0008

Absolute organ weights - group mean values (g) for animals killed after 4 weeks of treatment

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
THYMUS								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.486	0.503	0.496	0.464	0.534	0.456	0.450	0.528
sd :	0.077	0.094	0.098	0.106	0.101	0.101	0.111	0.091
THYROIDS+PARAS								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	9	9
MEAN :	0.012	0.012	0.011	0.012	0.011	0.010	0.011	0.011
sd :	0.004	0.004	0.004	0.003	0.004	0.004	0.002	0.003
UTERUS+CERVIX								
					BEHREN'S - FISHER'S TEST			
N :					10	10	10	10
MEAN :					0.52	0.52	0.52	0.40
sd :					0.17	0.23	0.24	0.08

TABLE 7B

Print No: 0009

Organ weights relative to bodyweight - group mean values (%) for animals killed after 4 weeks of treatment

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
TERMINAL BODY WEIGHT (g)								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	324.1	331.6	331.3	324.1	241.9	236.2	238.5	240.1
sd :	27.6	24.7	18.7	35.1	20.1	14.9	16.9	11.4
BRAIN								
	BEHREN'S - FISHER'S TEST				BEHREN'S - FISHER'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.609	0.600	0.593	0.608	0.799	0.836	0.803	0.793
sd :	0.054	0.033	0.022	0.056	0.048	0.070	0.056	0.025
EPIDIDYMIDES								
	DUNNETT'S TEST							
N :	10	10	10	10				
MEAN :	0.2377	0.2425	0.2502	0.2422				
sd :	0.0134	0.0258	0.0253	0.0238				
HEART								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.390	0.362	0.380	0.380	0.405	0.421	0.396	0.398
sd :	0.025	0.037	0.023	0.030	0.036	0.043	0.035	0.041
KIDNEYS								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.747	0.766	0.729	0.733	0.801	0.764	0.810	0.780
sd :	0.063	0.052	0.047	0.045	0.051	0.054	0.050	0.048

TABLE 7B - continued

Print No: 0009

Organ weights relative to bodyweight - group mean values (%) for animals killed after 4 weeks of treatment

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARO-1)
Dosage (mg/kg/day) : 0 500 1500 5000

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Schedule number: GSB 061

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
LIVER								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	4.18	4.34	4.33	4.12	4.40	4.09 a	3.96 b	4.28
sd :	0.33	0.41	0.26	0.34	0.19	0.30	0.26	0.23
LUNGS & BRONCHI								
	DUNNETT'S TEST				BEHREN'S - FISHER'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.438	0.432	0.454	0.461	0.504	0.519	0.516	0.510
sd :	0.049	0.031	0.030	0.034	0.030	0.080	0.022	0.023
OVARIES								
					DUNNETT'S TEST			
N :					10	10	10	10
MEAN :					0.0363	0.0339	0.0344	0.0357
sd :					0.0065	0.0047	0.0035	0.0053
SPLEEN								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.2135	0.2113	0.2431a	0.2236	0.2323	0.2185	0.2161	0.2202
sd :	0.0302	0.0225	0.0164	0.0294	0.0337	0.0413	0.0286	0.0281
TESTES								
	DUNNETT'S TEST							
N :	10	10	10	10				
MEAN :	0.988	0.978	1.023	0.985				
sd :	0.094	0.083	0.051	0.071				

Significant when compared with Group 1: a - $p<0.05$; b - $p<0.01$

Organ weights relative to bodyweight - group mean values (%) for animals killed after 4 weeks of treatment

Group : 1 2 3 4
Compound : Control Aspergillus niger (ARD-1)
Dosage (mg/kg/day) : 0 500 1500 5000

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
THYMUS								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	10	10
MEAN :	0.1507	0.1510	0.1503	0.1423	0.2200	0.1926	0.1874	0.2197
sd :	0.0263	0.0215	0.0317	0.0236	0.0311	0.0381	0.0386	0.0333
THYROIDS+PARAS								
	DUNNETT'S TEST				DUNNETT'S TEST			
N :	10	10	10	10	10	10	9	9
MEAN :	0.0038	0.0036	0.0034	0.0038	0.0045	0.0042	0.0044	0.0047
sd :	0.0012	0.0011	0.0012	0.0008	0.0014	0.0017	0.0009	0.0011
UTERUS+CERVIX								
					BEHREN'S - FISHER'S TEST			
N :					10	10	10	10
MEAN :					0.221	0.221	0.223	0.168
sd :					0.091	0.095	0.106	0.035