Diet: High Fat - Low Calcium (to mimic the "Western-style" diet)
A 6-Month Interim Report of a 9-Month Dietary Admixture Study of Ro 18-0647/008 (Orlistat) with Wistar Rats Using a High Fat, Low Calcium Diet (Study 06791): Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ 07110 Report 138945 dtd Oct 1996. Bulk Drug Lot: 950024 Q.A. - Present

This is an interim report on findings after 3 and 6 months of treatment with Ro 18-0647/008 and the associated recovery groups. A full report is to be issued at the completion of the study.

[This study was conducted concurrently with a study (No. 06806, GCR N-138946) using a high fat, normal calcium diet.]

Diet: Rat maintenance diet providing ca 40% of the calories from fat with a low (ca 0.1%) calcium content.

Dose: 0 (control), 70, 140, and 280 ppm Ro 18-0647/008 [Groups A, B, C, D] (0, 0.38, 0.78, 1.55 mg/gram Dietary Lipid) [From the final report these doses represent ca 2.8, 6.3 and 14.3 mg/kg/day for males and ca 4.0, 9.2 and 21.9 mg/kg/day for females.]
[Doses selected were expected to provide a range of inhibition of dietary fat absorption of ca 15% for the low dose and ca 70% for the high dose. - Reported to bracket the average inhibition of fat observed in humans given the therapeutic dose of Orlistat (120 mg t.i.d.).]

No. of Animals: 52 rats/sex/group Charles River (Crl:W)BR

Interim Sacrifices: 8/sex/group at 13 and 26 weeks. [Terminal sacrifice of 10/sex/group after 39 weeks of treatment.]

Recovery: At each sacrifice, an equal number of rats were randomly selected to be untreated for at least 8-weeks followed by sacrifice. [For Computer entry - Recovery Groups: 13 week - Study 06866; 26 week - Study 06867; path data reported as Study 06791.]

Pathology Evaluation: A full macroscopic examination was performed on all rats at necropsy, a limited list of tissues were collected (Adrenal glands, cecum, colon, duodenum, esophagus, heart, ileum, jejunum, kidneys, liver, lungs, pancreas, rectum, spleen, stomach, gross lesions) some of which were examined microscopically (Adrenal glands, Cecum, Colon, Duodenum, Ileum, Jejunum, Rectum, Gross lesions). Pathology included routine microscopic examination of multiple levels of the GI tract; quantification of cell proliferation in colons stained for proliferating cell nuclear antigen (PCNA), and examination of colonic whole-mount preparations for aberrant crypt foci and putative preneoplastic lesions.

In-life observations and determinations included clinical signs, body weight, and food consumption.

Treatment was well tolerated at all doses for 26 weeks. Treatment-related findings included unkempt anogenital and/or abdominal regions for some rats in each dose group and all treated groups showed decreases in body weight gain and and increased food consumption. Treatment-related changes showed a rapid reversal upon stopping treatment. Intestinal parameters evaluated showed no treatment-related effects. There were no treatment-related changes in the incidence or severity of aberrant crypt foci or intestinal histopathologic changes and there was no evidence of increased colonic cell proliferation.

Mortality: There were no apparent treatment-related mortalities.
(One 140 ppm male rat sacrificed in a moribund condition day 162.)

Clinical Signs: Intermittent unkempt anogenital and/or abdominal regions were seen in 1 low dose female, 2 mid-dose males and most high dose rats. During

recovery this sign disappeared promptly.

Other findings included alopecia, and crust around the eyes in most groups. 1M;1F control and 1F mid-dose had clonic convulsions during treatment as did 1M mid-dose during 13 week recovery period. One mid-dose F had a palpable mass.

Bodyweight: Treated rats showed dose-related decreases in bodyweight gain during the treatment period. Compared to controls there was a rapid gain of weight when treatment was stopped.

Mean body weights at day 182 low-high dose for males were 97, 95 and 88% of controls and for females 94, 89 and 87% of controls.

Mean body weight gain was significantly less than controls during this period in mid and high dose males being 39 and 30% gain vs 45% for controls and 42% for the low dose. At this same time period, female mean body weight gain was also significantly less than controls being 42, 35, 31% low-high dose vs 49% for controls.

Food Consumption: Increases in food consumption seen throughout the 26-week treatment period were dose-related. During the recovery period food consumption returned to near control levels. By day 182 low, mid, high dose food consumption was 117, 126 and 134% of controls for males and 108, 117, 142% for females.

Gross Pathology: There were no apparent treatment related findings. Tissue masses/neoplasms were varied and few and not apparently drug-related.

Histopathology: Findings were comparable with controls. Common incidental findings consisted of vacuolation of the adrenal cortex, progressive nephropathy involving kidneys and edema or hemorrhage involving the rectum and colon.

According to the sponsor, no treatment-related changes were seen in any of the intestinal parameters evaluated. There was no evidence of treatment-related changes in the incidence or severity of aberrant crypt foci or intestinal histopathologic changes and there was no increase in colonic cell proliferation at sacrifices after 13 or 26 weeks of treatment nor after the recovery periods (at 21 and 34 weeks).

Colonic cell proliferation (PCNA stain):

Because of the small percentage change, lack of dose proportionality and inconsistency of changes among colon sections, the sponsor reports that there were no differences in labeling index (LI) crypt height, or crypt grade that were considered to be related to treatment for any of the sacrifice periods. Statistical significance was $p<0.05$, unless otherwise noted. Colon section orientation: Proximal - A, D, B, E, C, F - Distal. Sections D and E were returned to fixative. Section F (adjacent to rectum) was to be evaluated only if other colon sections proved unsatisfactory.

First interim sacrifice (13-weeks):

The mean LI for all groups, males and females, ranged from 47.0 - 49.6% with no statistical significance. Mean crypt heights for all groups ranged from 27.2 to 31.4. A statistically significant increase in crypt height was seen in 70 ppm males and a decrease was seen in 140 ppm females. There was a lack of statistical significance for mean crypt grades for colon sections (A, B, C) which ranged from 3.4 to 4.2.

First interim recovery sacrifice (21 weeks):

The mean LI ranged from 48.5 to 52.4% for males and females of all groups. The mean LI for 280 ppm males and 70 ppm females was significantly greater than that for controls. The mean crypt height which ranged from 23.6 to 30.5 was significantly less for 280 ppm males and all females. Colon sections (A, B, C) had mean crypt grades of 3.3 to 4.1; that for section C in 280 ppm males was significantly less than that for controls.

Second interim sacrifice (26-weeks):

Mean LI for all groups of males and females ranged from 43.3 to 43.8% with no statistically significant differences. Mean crypt heights which ranged from 36.5 to 40.0 were significantly greater ($p<0.01$) for 280 ppm males compared to controls. Mean crypt grades ranged from 3.3 to 4.2 for colon sections A, B, and C. Compared to controls colon sections A and C for 280 ppm males were statistically different.

Second interim recovery sacrifice (34 weeks):

The mean LI for all group males and females ranged from 42.6% to 43.2% and was without statistical significance. Mean crypt height ranged from 30.7 to 32.8 and was significantly greater for 70 and 140 ppm females compared to controls. Colon sections A, B, and C had mean crypt grades ranging from 3.4 to 4.0. For females of the 140 ppm group the mean crypt grade for colon section C was significantly greater than controls.

Colonic aberrant crypt foci (methylene blue stain):

Second interim sacrifice (26 weeks) - The sponsor did not consider any differences in the incidence of aberrant crypt foci (ACF) to be treatment-related. For control through high dose the number of ACF in males was 1, 3, 6, 1 and for females 0, 3, 6, and 3.

Second interim recovery sacrifice (34 weeks): It is stated that there were no differences in the incidence of ACF that were considered to be related to treatment. For controls through high dose the total number of ACF in males was 3, 1, 3, and 4; that for females was 9, 7, 7, and 1.

[NOTE: The following final report was just received and added to the above completed review.]

Diet: High Fat - Low Calcium (to mimic the "Western-style" diet)

Final 9-Month Report (continuation of above study): Dietary Admixture Study of Ro 18-0647/008 (Orlistat) with Wistar Rats Using a High Fat, Low Calcium Diet (Study 06791): Q.A. - Present Report 139068 dtd Feb 1997.

Dose: 0, 70, 140, 280 ppm Ro 18-0647/008 (0, 0.38, 0.78, 1.55 mg/gram Dietary Lipid) Doses were ca 2.8, 6.3 and 14.3 mg/kg/day for males and 4.0, 9.2 and 21.9 mg/kg/day for females.

Terminal Sacrifice: 10/sex/group after 39 weeks of treatment.

Results:

There were 9 unscheduled deaths or sacrifices 1M control, 1M;2F low dose, 2M mid-dose and 3M high dose. Diagnosis was undetermined except for malignant lymphoma in one low dose male, atrial thrombosis with cardiomyopathy in 1 high dose male, and adenocarcinoma in the prostate also in 1 high dose male.

In general the drug was well tolerated. Treatment-related findings including unkempt anogenital and/or abdominal regions in some animals from each dose group were reversed after cessation of treatment.

Other clinical signs included alopecia, and crust around the eyes in most dose groups. Clonic convulsions were seen in 1M;1F controls, 2M low dose, 2F mid-dose. 1M mid-dose also had tremors and clonic convulsions during the recovery period. Palpable masses were seen in 2 control females, 1 mid-dose female and 1 high dose male. One high dose male had a bloody discharge from its penis and one control male had red tinted urine.

Dose related decreases in bodyweight gain and increased food consumption seen in all treated groups were reversed after treatment was stopped. At day 273 mean body weights for males were 95, 95 and 87% of controls and for females 89, 82 and 79% of controls. Mean body weight gain for high dose males was significantly less than that of controls (41 vs 60% for controls) and for females all treated groups were significantly less than controls being 66, 54, and 49% vs 82% for controls. Food consumption, significantly greater than controls, by day 273 ranged from 108 to 139% of controls. For previously treated groups, food consumption declined during the recovery period.

Microscopic correlation with tissue masses/neoplasm findings at necropsy included controls - 1M abscess, 1F kidney adenoma, 1F mammary fibroadenoma; low dose - 1M adrenal adenoma and 1M malignant lymphoma; mid-dose - 1M pheochromocytoma, 1M acute inflammation of the glandular stomach and 1M liver hemangiosarcoma, 1F fibroadenoma and 1F kidney mesenchymal tumor; high dose - 1M abdominal cavity hemangiosarcoma, 1M mammary fibroadenoma, 1M prostate adenocarcinoma, and 1F kidney malignant mesenchymal tumor.

It is reported that no treatment-related findings were seen in any of the intestinal parameters evaluated; there were no treatment-related changes in incidence or severity of aberrant crypt foci or intestinal histopathologic changes. There was no evidence of increased colonic cell proliferation. However, at the final 39 week sacrifice there was a treatment-related significant increase ($p<0.05$) in crypt height for high dose females when compared with controls. The mean crypt grades for colon section A of mid-dose females was significantly less than that of controls. However, the mean crypt grade for colon section C of high dose females was significantly greater ($p<0.05$) than that of the control group.

At the end of the recovery period, the mean crypt height and mean crypt grades of the low dose were significantly less than that of controls.

Crypt height was used to complement routine histopathology in the determination of mucosal hyperplasia (routine histopathology, however, was normal.)

At the final sacrifice there appeared to be a mild trend in improvement of aberrant crypt foci (ACF) parameters of treated vs control.

From Sponsor's Table:

The cumulative incidence of ACF:

		Males				Females		
Sacrifice Interval	Dose (ppm) 0	70	140	280	0	70	140	280
2nd Interim Sacrifice (26 wks)	1*	3	6	1	0	3	6	3
2nd Interim Recovery Sac(34 wks)	3	1	3	4	9	7	7	1
Final Sacrifice (39 wks)	10	5	2	1	16	13	14	1
Final Recovery Sac (48 wks)	7	4	9	2	15	14	6	7
TOTAL	21	13	20	8	40	37	33	12

*Total number of ACF/group. N= 5 rats/group at 26 and 34 weeks; N~10 rats/group at 39 and 48 weeks.

Diet: High Fat - Normal Calcium

A 6-Month Interim Report of a 9-Month Dietary Admixture Study of Ro 18-0647/008 (Orlistat) with Wistar Rats Using a High Fat, Normal Calcium Diet (Study 06806): Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ 07110 Report 138946 dtd Oct 1996. Bulk Drug Lot: 950024 Q.A. - Present

This is an interim report on findings after 3 and 6 months of treatment with Ro 18-0647/008 and the associated recovery groups. A full report is to be issued at the completion of the study.

[This study was conducted concurrently with a study (No. 06791, GCR N-138945) using a high fat, low calcium diet.]

Diet: Rat maintenance diet providing ca 40% of the calories from fat with a normal (ca 1%) calcium content.

Dose: 0 (control), 70, 140, and 280 ppm Ro 18-0647/008 [Groups A, B, C, D] (0, 0.38, 0.78, 1.55 mg/gram Dietary Lipid) [From the final report these doses represent ca 2.8, 6.7 and 15.3 mg/kg/day for males and ca 4.4, 10.0 and 22.1 mg/kg/day for females.]
[Doses selected were expected to provide a range of inhibition of dietary fat absorption of ca 15% for the low dose and ca 70% for the high dose. - Reported to bracket the average inhibition of fat observed in humans given the therapeutic dose of Orlistat (120 mg t.i.d.).]

No. Of Animals: 52 rats/sex/group Charles River (Crl:W)BR Rats

Interim Sacrifices: 8/sex/group at 13 and 26 weeks. [Terminal sacrifice of 10/sex/group after 39 weeks of treatment.]

Recovery: At each sacrifice, an equal number of rats were randomly selected to be untreated for at least 8-weeks followed by sacrifice.
[For Computer entry - Recovery Groups: 13 week - Study 06864; 26 week - Study 06865; path data reported as Study 06806.]

Pathology Evaluation: A full macroscopic examination was performed on all rats at necropsy, a limited list of tissues were collected (Adrenal glands, cecum, colon, duodenum, esophagus, heart, ileum, jejunum, kidneys, liver, lungs, pancreas, rectum, spleen, stomach, gross lesions) some of which examined microscopically (Adrenal glands, Cecum, Colon, Duodenum, Ileum, Jejunum, Rectum, Gross lesions). Pathology included routine microscopic examination of multiple levels of the GI tract; quantification of cell proliferation in colons stained for proliferating cell nuclear antigen (PCNA), and examination of colonic whole-mount preparations for aberrant crypt foci and putative preneoplastic lesions. [Due to error at 26-week interim sacrifice only tissues listed as examined microscopically were collected.]

Results:

In-life observations and determinations included clinical signs, body weight, and food consumption.

Treatment was well tolerated at all doses for 26 weeks. Treatment-related findings included unkempt anogenital and/or abdominal regions for some rats in each dose group and all treated groups showed decreases in body weight gain and and increased food consumption. Treatment-related changes showed a rapid reversal upon stopping treatment. Intestinal parameters evaluated showed no treatment-related effects. There were no treatment-related changes in the incidence or severity of aberrant crypt foci or intestinal histopathologic changes and there was no evidence of increased colonic cell proliferation.

Mortality: There were no apparent treatment-related mortalities. However, there were 13 unscheduled deaths or sacrifices (mostly in the control and 140 ppm groups) during the treatment and recovery periods.

Clinical Signs: Treatment-related signs limited to unkempt anogenital and/or abdominal regions of a few rats in all dose groups (greater in high dose - 4M;8F) were intermittent and disappeared promptly in recovery animals. Other findings included alopecia and crust around the eyes. Clonic convulsions were seen in 1 low and 1 mid-dose females and in 2 high dose males - reported as observed in a similar frequency in controls of this strain.

Bodyweight: Compared to controls treated rats showed dose-related decreases in body weight gain during treatment with a rapid gain during the recovery phase. Compared to controls at day 182, mean bodyweights (low to high dose) of males were 98, 95, and 89% of controls and for females 95, 94, 91% of controls. During this period mean bodyweight gain for mid- and high dose males was (33 and 24% gain vs 39% for controls) significantly less than that for controls. For this time period female bodyweight gain for all treated groups was significantly less than that for controls [32, 29, 25% (low to high dose) vs 38% for controls]. At the end of the 13 week recovery period male mean body weight gains were significantly greater than that of controls. Although mid and high dose females gained more weight than controls during this recovery period, only the high dose was significantly greater. Recovery after 26-weeks treatment showed mean bodyweight gain to be significantly greater than controls for mid and high dose males and high dose females.

Food Consumption: The treatment period showed dose-dependent increases in food consumption which returned to near control levels during the recovery periods. At day 182 food consumption, low-high dose, for males was 114, 122, and 131% of controls and for females 110, 121, and 132% of controls.

Necropsy: No apparent treatment-related findings were noted at necropsy. Kidney and urinary bladder showed the most frequently observed lesions found in control and treated. These incidental gross findings consisted of cysts, pelvic dilation, and granular surfaces in kidneys and calculi in urinary bladder.

Histopathology: Histopathological findings for treated were similar to that of controls. Vacuolation of the adrenal cortex was the most consistent finding observed in controls and treated males (nearly all males; also a small number of females). Adrenal cortex vacuolation was characterized by multifocal to diffuse, round, clear, cytoplasmic vacuoles in the zona fasciculata and zona reticularis. Other common lesions which the sponsor considered incidental included mineralization, pelvic dilation, and progressive nephropathy involving the kidneys and edema, hemorrhage, and dilated crypts in the intestines.

In addition it is reported that there was no evidence of increased colonic cell proliferation at 13 or 26 weeks or following their recovery periods at 21 and 34 weeks.

Colonic cell proliferation (PCNA stain):

The sponsor reports that because of the small percentage change and lack of dose proportionality there were no differences in labeling index (LI) crypt height, or crypt grade that were considered to be related to treatment for any of the sacrifice periods. Statistical significance was $p<0.05$, unless otherwise noted. Colon section orientation: Proximal - A, D, B, E, C, F - Distal. Sections D and E were returned to fixative. Section F (adjacent to rectum) was to be evaluated only if other colon sections proved unsatisfactory.

First interim sacrifice (13-weeks):

The mean male and female LI ranged from 46.8 to 48.7. Colon section A, B, and C crypt grades ranged from 3.4 to 4.1. The mean crypt grade for colon section C for 140 ppm males was significantly less ($p < 0.01$) than that for controls.

First interim recovery sacrifice (21 weeks):

A range of 48.9 to 51.5% was recorded for the LI of males and females from all groups. Males of the 70 ppm group showed a LI significantly less than that for controls - not considered by the sponsor to be treatment-related due to the small change and lack of dose proportionality. Mean crypt grades ranged from 3.1 to 4.1 for colon sections A, B, and C. For males of the 280 ppm group the mean crypt grade of colon section C was significantly less ($p<0.05$) than that of controls.

Second interim sacrifice (26 weeks): The mean LI for males and females ranged from 43.7 to 45.9% for all groups. The mean LI for females of the 280 ppm group was significantly less than that of controls. Mean crypt grades ranged from 3.3 to 4.2 for colon sections A, B, and C; none of the differences were statistically significant at $p<0.05$.

Second interim recovery sacrifice (34-weeks):

There was no statistically significant difference in mean LI's which ranged from 43.4 to 44.1% for males and females of all groups. Mean crypt heights ranged from 28.5 to 32.5 cells. 70 ppm males had crypt heights that were significantly less than that of the control group. Colon sections A, B, and C had mean crypt grades which ranged from 3.0 to 4.0. Colon section A for 140 ppm males had a mean crypt grade which was significantly less than that for controls.

Colonic aberrant crypt foci (methylene blue stain):

Second interim sacrifice (26 weeks): The incidence of aberrant crypt foci (ACF) were reported to show no differences considered to be treatment-related. For controls through high dose the total number of ACF was 3, 7, 1, and 0 for males and 5, 6, 3, and 8 for females.

Second interim recovery sacrifice (34 weeks): Reportedly there were no treatment-related differences in the incidence of ACF. For males, control through high dose, the total number of ACF was 8, 8, 3, and 1 and for females 6, 8, 15, and 10. Compared to controls there was a dose-related decrease in ACF for males and a dose related increase for females. According to the sponsor, these differences in total number of ACF/group were the result of differences in both the percentage of rats with at least one ACF and the number of ACF/rat. The combined incidence of ACF/group control through high dose was 11, 15, 4, 1, for males and 11, 14, 18, 18 for females. The meaning and potential significance of the dose-related decrease in ACF for males and dose-related increase for females is unknown. There were no neoplastic lesions noted in the GI tracts of any rats in this study.

[NOTE: The following final report was just received and added to the above completed review.]

Diet: High Fat - Normal Calcium

Final 9 Month Report (continuation of above study): Dietary Admixture Study of Ro 18-0647/008 (Orlistat) with Wistar Rats Using a High Fat, Normal Calcium Diet (Study 06806): Q.A. - Present

Dose: 0, 70, 140, 280 ppm (ca 2.8, 6.7 and 15.3 mg/kg/day for males and 4.4, 10.0 and 22.1 mg/kg for females) (0, 0.38, 0.78, 1.55 mg/gram Dietary Lipid)

Terminal sacrifice - 10/sex/group after 39 weeks of treatment.

Results:

Mortality (21) control - high dose: 3M;2F, 2F; 3M;5F, 2M;4F.

Controls - Males(3); 1 malignant lymphoma, 2 undetermined.
Females(2); 1 mammary adenocarcinoma, 1 kidney mesenchymal tumor.

Low dose - Females(2) 1 mammary adenocarcinoma metastatic to lung, 1 undetermined.

Mid-dose - Males(3) 2 undetermined, 1 progressive nephropathy with skin ulceration.
Females(5) 2 undetermined, 1 malignant lymphoma, 2 pyelonephritis.

High dose - Males(2) undetermined.
Females(4) 3 undetermined, 1 progressive neuropathy.

In-life Treatment-related Findings:

Unkempt anogenital and/or abdominal regions were seen in some animals from each dose group.

Dose-related decreases in bodyweight gain in treated groups. At the end of treatment body weights were 94, 91 and 86% of controls for males and 94, 88 and 87% for females. Mean bodyweight gain was significantly less than controls for male mid and high dose rats (43 and 36% gain vs 54% for controls). At the end of study female bodyweight gain was significantly less than controls in all treated groups (47, 40, 38% vs 59% for controls). Recovery rats showed a rapid bodyweight gain.

Increased food consumption was seen in all treated groups, ranging from 103-127% of controls at day 273. These changes decreased during the recovery period.

Other clinical signs considered by the sponsor to be unrelated to treatment included alopecia, crust around the eyes which were observed in most dose groups. Clonic convulsions observed in one low and one mid-dose female and two high dose males were reported to have been observed in control rats of this strain with a similar frequency.

No findings attributable to treatment were reported at necropsy. Incidental lesions were found in control and treated kidneys and urinary bladder which consisted of cysts, pelvic dilation, and granular surfaces in kidneys and calculi in the urinary bladder.

According to the sponsor, there were no treatment-related histopathology findings and no intestinal histopathologic changes or any evidence of increased colonic cell proliferation. However, vacuolation of the adrenal cortex observed in control and treated was present in nearly all males and in a small number of females. Other lesions included mineralization, pelvic dilation, and progressive nephropathy involving the kidneys and edema, hemorrhage, and dilated crypts in the intestines.

For the final sacrifice it is reported that there were no differences in mean labeling index, crypt height, or crypt grades considered to be treatment-

related. However the mean crypt height for high dose females was significantly greater than the control group. At the 48 week recovery sacrifice the mean labeling index of high dose males was significantly less than that of controls.

There was a **treatment-related increase** in the **number of colonic aberrant crypt foci (ACF)** noted in the mid- and high dose females at sacrifice after the 39 weeks treatment period being 8, 9, 18 and 50 control- high dose [vs 9, 12, 7, 7 for males]. Findings after the recovery sacrifice (48 weeks) were 10, 32, 38 and 16 for females and 4, 8, 8, and 7 for males. (See Table below.)

The increase in the number of ACF was in general not accompanied by an increase in ACF size [as measured by multiplicity or percentage of large ACF (ACF with $\geq$ 4 aberrant crypts) or findings of any of the other colon parameters evaluated. Male rats did not show comparable effects. There was no increase in ACF in the high fat/normal calcium parallel study in which the dietary calcium level was 10-fold less.

Since Ro 18-0647/008 is not genotoxic or carcinogenic in rats, the sponsor states that the clinical relevance of this finding is uncertain.

From Sponsor's Table:

The combined incidence of ACF:

		Males				Females		
Sacrifice Interval	Dose (ppm) 0	70	140	280	0	70	140	280
2nd Interim Sacrifice (26 wks)	3*	7	1	0	5	6	3	8
2nd Interim Recovery Sac(34 wks)	8	8	3	1	6	8	15	10
Final Sacrifice (39 wks)	9	12	7	7	8	9	18	50
Final Recovery Sac (48 wks)	4	8	8	7	10	32	38	16
TOTAL	24	35	19	15	29	55	74	84

*Total number of ACF/group. N= 5 rats/group at 26 and 34 weeks; N~10 rats/group at 39 and 48 weeks.

Dog - Diet: High Fat - Low Calcium

Two-week oral (feed admix) Toxicity Study of Ro 18-0647/008 in Male Beagle Dogs Fed a High-Fat/Low Calcium Diet: F. Hoffmann-La Roche Ltd., Basel, Switzerland. Report B-164'931 dtd. Nov 1995. Protocol 049 P 95. Batch 3084028 Q.A. - Present

Dose: 0, 0.3, 1.0, 3.0, 9.0 mg/kg/day (Groups A-E) for 17 or 18 days in the diet [calculated levels received: 0, 0.32, 1.0, 3.17, 9.33 mg/kg/day]

No. of Animals: 3 male beagle dogs per group

Pilot study to assess the effect of Ro 18-0647 when it is administered in conjunction with a diet high in lipids and low in calcium: The **diet** used in this study contained 16.77% (w/w) lipids and 0.14% (w/w) calcium. [Compared to that, the normal maintenance diet for dogs (Kliba No. 335) contains 5.8% (w/w) crude fat and 1.8% (w/w) calcium.]

Results:

Mortality: There were no deaths.

Clinical Signs: No drug-related signs were noted. However, individual dogs occasionally showed abnormally formed or red stained feces.

Bodyweight: no drug-related changes seen. At day 17 mean body weights compared to day 0 control through high dose were as follows: 98.9, 100, 95.8, 93.4, 91.3%.

Food Consumption: Two control dogs and 1 high dose dog did not consume their daily offered feed amount.

Hematology: No abnormal effects.

Blood Chemistry: Dose-related decreases were seen in serum cholesterol (by 18.2 to 47.4%) and in serum tocopherol (by 9.2 to 59.9%).

The increase in serum α-amylase in treated dogs of all test groups was probably the result of the high amount of fat in the diet. There was less of an increase in α-amylase in the high dose group probably due to the stronger inhibition of fat absorption.

The increased serum lipase activity seen in 1 Gp B, 2 Gp D and 1 Gp E dogs prior to the test period were further increased up to the end of the test period. This increase in serum lipase activity was not associated with any pancreatic histopathological changes.

Two Gp C and 1 Gp E dogs had an increased serum concentration of bile acids on day 15 - there was no correlation with other parameters or histopathological examination. [The fatty liver change in one of the C Gp dogs was only focal and according to the sponsor, not an explanation for elevated serum bile acids.]

Urine Analysis: Alkaline urine among all test groups including controls was reported as being due to the relatively high vegetable portion of the diet.

Organ Weights: [Adrenals, brain, heart (without atria), kidneys, liver, testes] No apparent drug-related organ weight changes were noted.

Necropsy - Histopathology: Reported that no drug-related findings were observed. Dilation of small intestine lymphatics noted in all dogs was slightly more pronounced in the ileum of 2 high dose dogs. The large intestinal crypts were dilated in some dogs of all groups. One control and all treated dogs showed sinusoidal dilation in the mesenteric lymph nodes which was more pronounced in treated. Kidneys of all dogs (including controls) showed tubular hyaline droplets which showed minimally increased severity in the mid and high dose groups.

Fecal Excretion:

Fecal calcium excretion decreased dose-dependently due to the increase of fecal dry weight.

Fecal fat excretion increased in a dose-related manner being 11.3, 21.7, 57.2, 65.9% low to high dose vs 7.7% for controls (calculated as a percentage of lipid intake). Triglycerides accounted for more than 80% of fecal fat at the two higher doses however, equal amounts of unesterified fatty acids were excreted at the lower two dose levels. There was approximately a 16 and 12 fold increase in the concentration of fecal diglycerides at the two higher dose levels. Excretion of fatty acids was biphasic with the largest increase being observed at 1 mg/kg. Except for the lowest dose level, bile acid excretion was reduced. Soluble FFA and BA did not follow effects on total concentration. Fecal water concentration of soluble FFA and BA concentration was slightly increased (ca 1.5 fold that of controls) at the low dose of 0.3 mg/kg/day. Soluble FFA tended to be lowered with higher dosage. There was no clear effect apparent on soluble BA concentration. However, the effect on total FFA was considerably larger with culmination at 1 mg/kg/day and total BA concentration was reduced, not enhanced. There was considerable variability between individual dogs which received the same treatment.

Two-Week (20 Day) Oral (Feed Admix) Pilot Toxicity Study in Dogs: F. Hoffmann-La Roche Ltd, Protocol 013P94. Research Report B-164'940 dtd Aug 1996. Batch 3034009. Q.A. - Present.

To assess the effect of Ro 18-0647/008 administered in conjunction with a diet high in lipids and to establish levels for a 1 year study. The dietary fat content used in feed (Kliba 30-3359) for both dose groups was 17.3% (w/w) or ca 40% of metabolizable energy as fat. [The normal maintenance dog diet contains ca 6% (w/w) of crude fat.] Doses of 250 and 1000 mg/kg (no controls) Ro 18-0647/008 were given in the feed for 1 hour (restricted access)in the morning daily to groups or 2 male and 2 female beagle dogs for 20 days. [Intended dose levels were exceeded by 2-27% for the low dose and 27-33% for the high dose. Vitamins A, D, E and K were administered s.c. once in week 1.

Reportedly, there were no deaths or clinical signs, body weight (slight weight losses of ca 5% in males and ca 10% in females) or hematological changes and no organ weight, necropsy or histopathological changes characteristic of Ro 18-0647/008 treatment. [It is reported that an additional 4-week pilot toxicity study in dogs (062 P 94; no report) was performed to clarify whether administration of Ro 18-0647/008 at dose levels of 250 and 1000 mg/kg/day mixed in diets containing 30 or 40 cal% fat inhibits the body-weight development. No effect on the bodyweight was observed in that study.]

Mean food consumption was increased for both dose groups.

Compared to mean pre-test values (no controls) there were increases in plasma urea (18-34%) and decreases in plasma cholesterol (by 48 to 73%), plasma tocopherol (by 44 to 64%) and plasma retinol by (3 to 47%). A difference in postprandial mean plasma triglyceride was noted at 1 hr (low greater than high dose) and 6 hrs (high exceeded low dose), but not at 3 hrs, after feeding.

Urinary pH of both doses tended to become less alkaline.

Maximum plasma concentrations (generally at 1-3 hrs) of unchanged Ro 18-0647 ranged from 435 to 1600 ng/ml for the low dose and 1380 to 3550 ng/ml for the high dose. Most of the high dose test dogs were continuously exposed to the unchanged drug during the two-weeks of testing. $AUC_{(0-24hrs)}$ were in the range of 2500 to 11700 and 9690 to 26200 ng•h/ml for the 250 and 1000 mg/kg/day dose groups, respectively. Exposure was similar for both sexes and slightly less than dose proportional in both genders. The systemic exposure of unchanged Ro 18-0647 decreased during the 2 weeks of treatment. [The present data show that administration of the test drug as a feed admixture, with only 1 hour/day access to food, greatly reduced the intra- and inter-subject variability.]

There was a marked increase of fecal lipid excretion observed in both groups (between 63 and 80% of lipid intake).

One Year Oral(FA) Toxicity Study of Ro 18-0647/008 in Beagle Dogs: F. Hoffmann-La Roche Ltd., Basel, Switzerland. Research Report B-164'936 dtd. June 1996. Study Protocol 116 P 94. Batch (1) 3044013, (2) 3044014 Q.A. - Present

The object of this study was to assess the effect of Ro 18-0647 when administered in conjunction with a diet high in lipids. Doses were selected on the basis of the pilot studies 013 P 94 (20 day study) and 062 P 94 (28-day study).

Diet: The dietary fat content used in feed for control group B as well as dose groups C, D and E was 17.3% (w/w). The normal maintenance diet used for control group A contained 4.7% (w/w) of crude fat.

Vitamins: Vitamins A, D, E and K were orally or intramuscularly administered once to five times per week.

Dose: 0, 0, 10, 100, 1000 mg/kg (Groups A-E)
[Control Group A - standard diet; Control Group B - high fat diet.]

No. of Animals: 4M and 4F per group (additional 2M;2F in high dose group)

Recovery - 2M;2F high dose only for a period of 13 weeks

Results:

Reported that there were no clinical signs, no body-weight or organ-weight changes as well as no ophthalmological, electrocardiographic, hematological, necropsy or histopathological findings attributable to Ro 18-0647/008 treatment.

Mortality: There were no deaths.

Clinical Signs: None attributable to drug treatment.

Bodyweight: Mean bodyweight gains (mean wt day 371 minus day 0) as percents were as follows: Gp A ♂118.28, ♀117.24; Gp B ♂125.00, ♀117.50; Gp C ♂111.58, ♀112.20; Gp D ♂117.86, ♀103.57; Gp E ♂122.22, ♀113.75. Growth was most pronounced in male control Gp B as well as female control Gps A and B an less in the female mid-dose group.

Food Consumption: Compared to high-fat diet Control Group B, food consumption was increased in all dose group males by 49 to 74% and in females by 86 to 103% as follows: Groups B, C, D, E - Males = 100%, 174.23, 148.88, 172.11%; Females = 100%, 196.24, 203.18, 185.65%. Reported that the pharmacological effect of inhibiting pancreatic lipase indirectly decreased the caloric value of the feed admixtures. The dosed dogs compensated for the indirect reduction of the fat energy by an increased intake of feed admixture.

Ophthalmoscopic Exam: No treatment-related findings were seen.

Electrocardiogram: No findings attributable to drug treatment were observed.

Hematology: Differences were considered by the sponsor to be incidental and of normal biological variation.

Blood Chemistry: [Compared to Control Group B (high-fat diet)]
There was an increase in plasma urea (males by 33 to 63%; females by 34 to 39%) and decreases in plasma cholesterol (males by 49 to 69%; females by 56 to 73%; dose-related in both sexes); plasma cholecalciferol (males by 57 to 65%; females by 42 to 63%); plasma tocopherol (males by 77 to 81%; females by 81 to 83%); liver retinol (males by 50 to 88%; females by 49 to 57%) and liver tocopherol (males by 73 to 80%; females by 71 to 86%). Except for the decrease of liver retinol, other decreases were absent following withdrawal of treatment.

Plasma triglycerides (postprandial) were decreased in the low dose group (Males by 6 to 44%; females 7 to 48%). Compared with the corresponding values of the low-dose group, plasma triglycerides (postprandial) were increased in the mid-dose group (males by 30 to 104%; females by 52 to 94%) and high dose group (males by 158 to 398%; females by 126 to 247%) during 1 to 7 hrs after the feeding period.

Urine Analysis: The urinary pH of treated dogs tended to become less alkaline than that of controls. This was probably due to increased feed (protein) consumption produced by the drug which could lead to a more acid urine.

Drug Intake: Intended dose levels were exceeded by 11 to 22%.

Organ Weights: No findings were reported to be due to Ro 18-0647/008 treatment.

Necropsy: No findings were reported to be attributable to drug treatment. Incidental spontaneous findings included: gray-white nodules and foci (<1 cm) in

the lungs of several dogs in various dose groups (except high dose females) and prominent mesenteric lymph vessels.

Histopathology: No findings were reported as attributable to drug treatment. Various findings reported as incidental and spontaneous were most common in the lung, intestinal mucosa, thyroid gland, kidney, skeletal muscle and sciatic nerve. Major findings included: lung - foci of chronic inflammation (granulomas); intestinal mucosa - epithelial vacuolation (small intestine of 11 dogs regardless of dose group); thyroid - C-cell hyperplasia; kidney - medullary mineralization and epithelial vacuolation (females only); skeletal muscle and sciatic nerve - chronic inflammation in the hind leg [none in Gps A & B; Gp C muscle 2M, sciatic nerve 1M; Gp D muscle 1M, sciatic nerve 2M,2F; Gp E muscle 2M, 2F(1F=recovery), sciatic nerve 1M.].

Toxicokinetic Data: Blood samples were collected on study day 1 and one day during weeks 5, 14, 26, 39 and 52 at 1, 3, 6-7 and 24 hrs post drug (control Gp B sampled only at 1 hr) and analyzed for Ro 18-0647 and metabolite Ro 42-3988. Most plasma concentrations in the low dose group were below the limit of quantitation. In addition interanimal variability was at least partly coming from interanimal differences in the actual drug intake.

There was continuous exposure to unchanged Ro 18-0647 during the entire study duration. For the high-dose group C_{max} ranged from 500 to 4000 ng/ml (generally achieved within 2-3 hrs after administration) and AUC values ranged from 4-5 to 50 µg.h/ml. For the mid dose group the mean C_{max} ranged from 20 to 270 ng/ml. Plasma concentrations of the Ro 42-3988 (M1) metabolite represented 10-30% of the corresponding concentrations of Ro 18-0647. [This study using feed admix of orlistat to dogs is reported to have considerably reduced the extremely large interanimal variability previously observed in the 1-year toxicity study in which orlistat was administered by means of gelatine capsules (GCR B-153'797). According to the Sponsor:

There was no accumulation but rather a trend towards a decline of systemic exposure with time. Systemic exposure in week 52 represented 20-30% of that observed the first day of treatment, most likely, according to the sponsor, as a result of decreased GI absorption of drug.

There were no major gender differences in systemic exposure, despite that in the 100 mg/kg dose group higher AUCs (1.5-2 fold) were generally observed in females over that of males over the 1 year treatment. Similar trends were observed for metabolite Ro 42-3988 (M1).

In several instances in female dogs there was a dose proportional systemic exposure in the dose range of 100-1000 mg/kg. In male dogs, the systemic exposure was more than dose proportional. With regard to overall systemic exposure metabolite Ro 42-3988 (M1) decreased with increasing dose administered from 20-30% to 10-20% of the corresponding AUCs for unchanged compound in the 100 and 1000 mg/dose groups, respectively. Such findings may indicate a dose dependent increase of GI absorption and/or saturation of the first-pass metabolism of the drug at these dose levels.

The sponsor concludes that since levels of Ro 18-0647 below 0.2 ng/ml were seen in plasma samples from obese patients treated with 120 mg t.i.d. for 12 months the present dog toxicokinetic data provides a safety factor far above 1000.

Fecal and Rectal Lipids: (assessed in weeks 5 and 53 of treatment)

All dose levels showed markedly increased fractional excretion of dietary lipid. At the two higher dose levels the increase in fecal lipid tended to be more marked in week 53 than in week 5 in males but not in females. Lipid