

Toxicokinetics: Group A blood samples taken from 3/sex/group mice at various time intervals from one 24 hr. period during week 96 were studied for the estimation of plasma levels of unchanged Ro 18-0647.

[Adapted from sponsor's tables] Mice

Dose (Mg/kg/day)	C _{max} (ng/ml)		AUC _(0-24 hrs) in ng·h/ml	
	Male	Female	Male	Female
25	NM*	NM	NM	NM
375	17.4	8.0	230 (1.7)**	90 (1.3)
750	31.4	22.5	503 (1.9)	307 (2.2)
1500	55.8	39.7	1030 (2.0)	803 (2.8)

* Not Measurable

** () = AUC_(week 95/105)/AUC_(week 2) Both 2 week and present data were normalized for a 1000 mg/kg dose.

Allocation Group B - Animals for Interim Sacrifice

Mortality: Survival was not affected by treatment.

Clinical Signs: There were no treatment-related clinical signs.

Bodyweight: Male and female body weights were not affected at doses up to 750 mg/kg. At 1500 mg/kg/day female (but not male) bodyweights were decreased by ca 8% during the first 3 weeks of study.

Food Consumption: All mice showed an increase in food consumption with a dose response between 25 and 750 mg/kg/day. For males the increase was 21-38% and for females 16-35%. Relative food consumption (g/kg body weight/day) also showed increases.

Organ Weights: Male and female organ weight changes were variable but not treatment-related. However, in general some non-significant increases were seen for high dose ovaries at 78 weeks.

Necropsy (combined interim sacrifices) - Gross Changes: Similar to controls. It was reported that there were no nodules, masses or any other gross anatomic changes noted at necropsy that were considered to be drug associated. For high dose females, the incidence of ovarian watery cysts was greater than that of controls (probably the cause of increased ovarian weights at this dose). No histopathology was performed on these animals.

Drug Intake: The mean intake of Ro 18-0647/008 varied from -0.4% to 12% of that of the intended dose in males and females.

Plasma Vitamin Levels after 4-Weeks of Dosing: Plasma levels of Vitamin A were not affected. However, plasma levels of Vitamin E and Vitamin D₃ showed a dose-related decrease. [Mice in the three highest dose groups had been supplemented orally with Vitamins E and D₃ once weekly.] Vitamin E levels control (2) through high dose for males were 12.28, 10.52, 4.84, 2.92, 2.78, 3.49 µmol/L and 14.32, 13.22, 6.76, 3.86, 4.40 and 4.02 µmol/L for females. Vitamin D₃ levels control (2) through high dose for males were 241.2, 258.8, 58.8, 21.0, 28.8, 28.0 nmol/L and 173.8, 151.2, 52.5, 30.6, 27.5, and 23.2 nmol/L for females.

Hepatic Levels of Vitamins A and E: Levels of Vitamins A and E were determined in liver samples collected from 3-6 mice/sex/group at the time of their scheduled interim sacrifice. Liver Vitamin A tended to increase for the high dose males

during the course of the study being 132% of controls after 78 weeks. High dose females showed increases during the first third of study. Findings for the 25 mg/kg group were similar to that of controls. Except for the high dose, findings were variable up to 26 weeks. Absolute hepatic Vitamin A values after 52 and 78 weeks in all groups, including controls, were markedly higher when compared with previous determinations. According to the sponsor, the increase in Vitamin A is attributed to an age-related accumulation (control and low dose groups) and/or the supplementation with fat soluble vitamins for the three highest dose groups.

Liver stores of Vitamin E were decreased in all treated groups even though the three highest dose groups received vitamin supplementation. Compared with controls hepatic Vitamin E concentrations (low thru high dose) after 78 weeks were ca 11, 24, 26, and 39% for males and ca 28, 18, 26 and 34% for females. [Values determined after 52 weeks for any group, including controls, were markedly higher when compared with previous determinations or the determination after 78 weeks. These findings are unexplainable.]

Toxicokinetics: Single plasma samples from 6/sex mice on one day during weeks 26, 52 and 78 were used for systemic exposure. At 25 mg/kg/day plasma levels of unchanged drug were below the level of quantitation of 10 ng/ml.

[Adapted from sponsor's table]

Weeks Of Dosing	Mice					
	375 mg/kg/day		Ro 18-0647 (ng/ml) 750 mg/kg/day		1500 mg/kg/day	
	Male	Female	Male	Female	Male	Female
26	6.6±8.0	NM*	16.5±5.2	NM	10.0±19.7	9.4±10.6
52	10.4±6.2	4.0±6.9	18.7±10.8	14.5±11.7	26.5±17.5	14.1±12.9
78	NM*	10.3±9.7	15.4±12.5	NM	46.5±15.3	32.8±56.8

* Not Measurable

There was a 1.3-2.8 fold increase of the systemic exposure over the 2-year treatment. (However, it is stated that all clinical studies indicated a lack of accumulation of orlistat in human plasma.) There was a dose proportional systemic exposure in male mice. For females the exposure was more than dose proportional in the dose range of 375-1500 mg/kg. Males showed 1.5-2 fold higher systemic exposure than females over the 2-year period. There was a large interanimal variability which resulted in a considerable overlap between dose groups.

Colonic Mucosal Proliferation: The colons of mice sacrificed after 4, 26, 52, and 78 weeks dosing were examined for cell proliferation by means of staining histological sections for proliferating cell nuclear antigen (PCNA) utilizing an immunohistochemical staining procedure. N = 5 crypts/mouse and 2-6 mice per group.

Mild increases in cell proliferation tended to increase with dose and duration of exposure to drug. The 1500 mg/kg/day male group showed a mild increase in cell proliferation (15-33%, p<0.05) after 26, 52, and 78 weeks. Females of this group showed a +34% increase (p<0.05) after 52 and 78 weeks of treatment. A significant increase in cell proliferation was also seen in 25, 375, and 750 mg/kg males after 78 weeks (17-38%, p<0.05) and in the 750 mg/kg females after 52 weeks (14% p<0.05).

Females but not males showed an increase of 35-55% in crypt height at 78 weeks. The effect in females on crypt height was statistically significant but

it was not dose-related and histology showed no hyperplasia.

Although effects were seen on colonic cell proliferation and crypt height, no evidence of increased risk for intestinal carcinogenesis was reported.

Fecal Fat Analysis: (Adapted from Sponsor's Table)

Dose (Mg/kg/d)	Fecal Fat Excretion During Week 70*				
	0	25	375	750	1500
Male	10.8(10.8)**	35.6(35.6)	42.3(49.6)	40.6(42.1)	35.7(49.1)
Female	7.8(8.3)	17.2(20.1)	34.6(35.5)	33.5(39.3)	31.7(39.3)

* Percent of dietary intake; mean for 7-10 control mice and 4-6 treated mice per dose group.

** Figures in brackets are mean group values when mice with excessive food spillage were eliminated from the calculation; for the eliminated mice, calculation of fat intake was uncertain. [After exclusion of those that spilled food, the number of animals remaining in each group was small.]

Composition of fecal lipid for the most part showed little change from control with respect to fatty acid, diglyceride, and triglyceride excretion.

Unesterified fatty acids accounted for about one third of the increase of fecal fat at the low dose; at the three high dose levels fecal fat was largely excreted as triglycerides (90% of total). The drug did not increase daily excretion of bile acids nor the fecal concentration of soluble bile acids present in the aqueous phase. At all dose levels the fecal concentration of unesterified fatty acids was increased. This effect was largest at the 25 mg/kg/day dose level (2-3 fold). Fecal concentration of diglycerides was increased for the three higher dose levels. Relative to controls (below limit of quantification) the concentration of diglycerides was increased at least 9 to 10-fold at the maximally effective doses of 750 and 1500 mg/kg/day. [Lipid excretion was reported to tend to plateau at the three largest dose levels (375, 750, 1500 mg/kg/day) at ca 40-50% in males and 30-40% in females relative to dietary intake.] Treated mice especially the lower dose, excreted an unusual lipid component (1-alkyldiacylglycerol) not seen in control feces. According to the sponsor, the low efficacy of Ro 18-0647 observed in this study relative to the inhibitory effect on fat absorption is unexplained.

APPEARS THIS WAY ON ORIGINAL

Oncogenicity (Feeding) Study with Ro 18-0647/008 - Polymorph B (Orlistat) in the Rat: [REDACTED] Project 319623; Hoffmann-La Roche Study 06087. Report N-138885 dtd Oct 1996. Study dates: Apr 92 - Apr 94. Lot Nos. (Bulk Drug) 26031, 26033, 26034, 2013008. Q.A. - Present
[Ro 18-0647/008 (polymorph B) designates a [REDACTED]]

Dose: 0, 0, 150, 500, 1000 mg/kg/day (reported as 8599 mg/M²/day) by dietary admixture (Groups 1-5)

Number of Animals: 50M;50F per dose (Allocation Group A)

Wistar, Hanover-derived, SPF quality Rat.

An additional 30 rats per group (Allocation Group B) were designated for interim sacrifice at 26 and 52 weeks (5/sex/group) and at 78 weeks (15/sex/group). (Remaining 5/sex/group rats for metabolic investigations.)

Diet: pelleted Kliba 05-343-7 (=Kliba 05-343-10) ca 22% of calories as fat and 0.98% of calcium

Vitamin Supplementation: Groups 3, 4, and 5 received fat soluble vitamin supplementation weekly by gavage. Groups 1, 2, received 0.4 ml/rat vehicle (peanut oil) only. Group 3 received 0.2 ml/rat, Groups 4 and 5 received 0.4 ml/rat. Vitamin supplement composition: Vitamin A - alcohol - 1,725 µg/ml; alpha-Tocopherol - 100, mg/ml; Vitamin D₃ - 7 µg/ml; Vitamin K₁ - 1,400 µg/ml; Beta carotene - 7 mg/ml. [Beginning week 27 dl-α-tocopherol changed to d-α-tocopherol.]

Results:

Allocation Group A - Oncogenicity Animals

Mortality: Survival reported to show no adverse treatment-related effects. There was a slightly increased incidence of intercurrent mortality noted in males of Groups 3 and 5, when compared with controls (not considered by the sponsor to be related to drug administration).

Clinical Signs: No signs of intolerance were noted.

Nodules/Masses: The drug did not show any treatment-related effects on the incidence or group distribution of palpable masses.

Bodyweight: Bodyweight gain of treated males was moderately to slightly decreased while that of females was slightly decreased. Mean bodyweight gain (control Gp 2, low, mid, high dose) relative to Group 1 controls was 101, 89, 82 and 81% for males and 96, 90, 93, 94% for females.

Food Consumption: There was a tendency towards a dose dependent increase in food consumption of treated rats. The mean percent increase in food consumption (low, mid, high dose) for male rats was 26, 26, 37% while that for females was 24, 29, 33%.

Drug Intake: Closely approximated the intended dose.

Hematology: [Differential blood counts on surviving control and high dose group] There were no treatment-related adverse findings.

Organ Weights: The low dose group was unaffected. Female mean adrenal weights of the mid and high dose groups were increased by 41% and 86%, respectively compared with Control Gp 1. Male mid and high dose kidney weights were increased 12% and 21%, respectively.

Histopathology:

Non-neoplastic Lesions: Both sexes of the two highest dose groups showed an increased incidence of diffuse adrenal cortical hypertrophy which was treatment-related. All treated females also showed an increased incidence of pulmonary alveolar histiocytosis and renal nephropathy which were treatment-related. The incidence and severity of all other non-neoplastic lesions were considered by the sponsor to be commonly diagnosed in rats of this age and strain.

Sponsor's table:

Dose (mg/kg/d)	Incidence of Non-neoplastic Lesions (n=50)					
	Adrenal Glands		Lungs		Kidneys	
	Male	Female	Male	Female	Male	Female
0	4	0	30	19	1	1
0	1	3	34	29	2	3
150	3	7	34	36	0	10
500	41	39	43	45	3	13
1000	46	47	37	43	0	13

Neoplastic Lesions: The incidence of mammary gland fibroadenomas was decreased and that of pituitary gland adenoma was increased (70 vs 51% in controls $p < 0.05$) in females of the high dose group. The type, incidence, and organ distribution of all other neoplastic lesions was not different from that of controls. Also similar to that of controls and/or commonly diagnosed in rats of this age and strain were the number of primary neoplasms, the number of rats with primary neoplasms, the number of rats with more than one primary neoplasm, the number of rats with metastases, and the number of benign and malignant neoplasms per group and sex.

Colonic/Rectal Mucosal Proliferation: The colon and rectum from all rats were examined for cell proliferation after 104 weeks of dosing. Histological sections were stained for proliferating cell nuclear antigen (PCNA) using an immunohistochemical staining procedure. The colon showed no treatment-related adverse effects. Minimally increased cell proliferation was seen in the rectum of male rats from the two higher dose groups.

Adapted from Sponsor's table:

Nominal Dose (mg/kg/day)		Crypt Grade (mean \pm S.D.)			
		Male Rats		Female Rats	
		Colon	Rectum	Colon	Rectum
0	N=10	3.6 \pm 1.4	3.7 \pm 0.8	4.5 \pm 1.4	4.8 \pm 1.1
150	N=5	3.2 \pm 1.1 (-11%)	3.8 \pm 1.1 (+3%)	5.0 \pm 0.7 (+11%)	4.8 \pm 1.3 (0)
500	N=5	3.4 \pm 0.9 (-6%)	5.2 \pm 0.4 (+40%)	4.0 \pm 1.0 (-11%)	5.2 \pm 0.8 (+8%)
1000	N=5	3.2 \pm 1.1 (-11%)	5.4 \pm 0.5 (+46%)	4.0 \pm 1.4 (-11%)	5.2 \pm 0.8 (+8%)

Figures in () are the % change from control

Toxicokinetics: [Systemic exposure to unchanged drug was determined in 3 rats/sex/time interval over a single 24 hr period during week 104 of study.] Sponsor's tables:

Nominal Dose (mg/kg/day)	Male Rats			Female Rats		
	C _{max} (ng/ml)	C _{min} (ng/ml)	AUC _{0-24hr} (ng·h/ml)	C _{max} (ng/ml)	C _{min} (ng/ml)	AUC _{0-24hr} (ng·h/ml)
150	133	BLQ*	1160	89.2	BLQ	988
500	1190	13.5	12300	1870	5.9	15700
1000	2270	148	24500	3570	166	29800

BLQ* = Below the Level of Quantitation

A comparison of the AUC's after 104 weeks of dosing with data generated in relatively young rats after two weeks of dosing showed unchanged orlistat to accumulate with time.

Nominal Dose (mg/kg/day)	AUC _(week 104) /AUC _(week 2)	
	Male Rats	Female Rats
150	5.0	3.1
500	5.2	5.0
1000	3.2	2.4

Fecal Fat Analyses: Pooled fecal samples were collected during Week 101 from 5 cages/group (3-5 rats/cage).

All dose levels showed fecal excretion of ca 60-70% of ingest fat with only minor differences between male and female rats. Approximately 80% of the increase in fecal fat was accounted for by triglycerides with diglycerides accounting for less than 8%. At 150 mg/kg the concentration of fecal unesterified fatty acids tended to increase ca 1.2 to 1.6 fold but it was decreased in the 1000 mg/kg/day group. According to the sponsor, this biphasic pattern likely represents increasing suppression of residual lipase activity in the lower bowel in response to dose escalation. Fecal water content was minimally affected suggesting little effect on water absorption in the large intestine.

Excretion of Radioactivity from ¹⁴C-Labeled Ro 18-0647: An investigation was carried out to assess any possible change in exposure (absorption) during long term treatment (2 yrs.) and any change in absorption in old versus young rats. Six male (2 control, 4 treated) and 5 female (2 control, 3 treated) 2 year old rats from parallel groups (500 mg) of the rat carcinogenicity study were treated with a single final dose of ~500 mg ¹⁴C-Ro 18-0647/0004 (Batch 12306.93.2; label in C-3 of oxetan moiety).

Urinary excretion of radioactivity for control male rats amounted to 2.0 to 5.3% of the administered dose vs 0.6 to 1.4% for treated males. That for female controls was about 1.2 to 2.4% and for treated females 0.8 to 1.3%. Fecal excretion of radioactivity for control males was 94.4 to 98.7% and that for treated males was 89.4 to 96.2%. For female controls the values were 92.7 to 94.5% and for treated females 91.8 to 96.7%. Minimal differences were evident in absorption in 2-year old rats following longterm treatment or treatment of young [urinary excretion taken as a measure of absorption (range 2.5 up to 10%)] versus old rats. [Urinary and fecal recovery of ¹⁴C was essentially complete in the first 72-96 hours.]

Allocation Group B (Rats Designated for Interim Sacrifice):

Drug Intake: Closely approximated the intended dose.

Mortality: There were no treatment-related adverse effects on survival.

Clinical Signs: There were no treatment-related signs of intolerance.

Nodules and Masses: Neither incidence nor group distribution showed any treatment-related effects.

Bodyweight: Males, but not females, showed some decrease in bodyweight gain (low to high dose = 88, 83, 86%). Males showed the first statistically significant deficit at 33 weeks for the high dose group.

Food Consumption: There was an increase in food consumption with a tendency towards dose dependency for treated males and females over the entire dosing period. Mean percent increase low thru high dose relative to control Group 1: Males 26, 27, 33%; Females 30, 32, 36%.

Hematology: Effects at 500 and/or 1000 mg/kg at mainly 78 and/or 52 weeks were related to drug. These changes included: RBCs - slight decreases in high dose M and F; Hb - slight decrease in high dose males. Mean Corp. Vol. - slight increase in M;F high dose and F low and mid dose; Mean Corp. Hb - slight increase in F all doses and M high dose; Mean Corp. Hb Conc. - slight decrease in male mid and high dose; Red Cell Distribution Width - slight increase in F high dose; Reticulocyte Count - slight increase in M;F mid and high dose; Reticulocyte Fluorescence Ratios - M;F mid and high dose slight increases in % Reticulocyte HFR and slight decreases in % Reticulocyte LFR; Differential Leukocyte Count - F mid and high dose slight decrease in segmented neutrophils and slight increase in lymphocytes in F high dose.

Blood Chemistry: [According to the sponsor, marked lipemia and interference with photometric measurements occurred in the two highest dose groups (primarily bilirubin, total cholesterol and triglycerides); in addition the diet was relatively high in protein.] Most of the changes that achieved statistical significance were found in the 500 and 1000 mg/kg groups. Most findings were at 52 and 78 weeks, however, some were also noted at the 26 week sacrifice. Findings included: Glucose - slight increases in M and F; Urea - slight increases in M at all treated doses and F mid-dose; Creatinine - slight increase in high dose males; Total Bilirubin - marked increases in mid- and high dose M and F; Total Cholesterol increases moderate in high dose males and slight in mid-dose females; Triglycerides - markedly increased in M and F; Aspartate Aminotransferase (ASAT) and Alanine Aminotransferase (ALAT) slight decrease in females; Gamma-glutamyltransferase (GGT) - moderate increases in M and F; Calcium - slight increase in F high dose; Phosphorus - slight increases in M and F; Sodium - slight decrease in females; Chloride - slight decreases in M and F; Total Protein - slight increases in M and F; Serum Protein Electrophoresis slight changes in M and F.

Hepatic Levels of Vitamins A and E: [Determined after 26/27, 52 and 78 weeks of dosing.] Vitamin A hepatic levels were either increased (25-36%) or unchanged in female rats or were slightly decreased ($\leq 25\%$) in male rats. Hepatic vitamin E levels were markedly decreased without regard to dose in all rats, at all doses, at each interim sacrifice. Findings at 26/27 weeks of dosing ranged from -81% to -82% for males and -57% to -68% for females; at 52 weeks -65% to -67% for males and 0 to -49% for females; at 78 weeks -64% to -73% for males and -56% to -69% for females.

Absolute Vitamin A levels were increased for all groups including controls after 78 weeks when compared with prior determinations.

Organ Weights: The low dose group showed no treatment-related adverse effects. Compared to controls, **adrenal glands** showed considerable non-dose related **increases** for males (<females) and females as early as the 26/27 week sacrifice. Increases compared to controls at 26/27, 52 and 78 weeks, respectively: high dose - males 26, 31, 29% and females 81, 77, 49%; mid-dose - males -, 37, 24% and females 44, 78, 53%. (changes based on at least one organ weight parameter.) See associated histopathological changes below.

Kidney weights of high dose males were **increased 17%** over that of controls at the 26/27 week sacrifice. [Not associated with histopathological change.]

Histopathology:

Non-neoplastic Lesions: The incidence of diffuse adrenal cortical enlargement (hypertrophy) Male, Female and Total was as follows: Control Group A - 0, 2, 2; Low dose group 1, 0, 1; mid-dose group 9, 14, 23; high dose group 14, 15, 29. The increased cell size was confined to the zona fasciculata and reticularis with severity at the high dose being greater than that at the mid-dose.

Neoplastic Lesions: The type, incidence, and organ distribution of neoplastic lesions was similar to that of controls.

Colonic/Rectal Mucosal Proliferation: Rats were sacrificed after 26/27, 52 and 78 weeks of dosing and the colon and rectum were examined for cell proliferation. Histological sections from 4-10 rats/sex/group were stained for proliferating cell nuclear antigen (PCNA) by an immunohistochemical staining procedure.

The colon showed no statistically significant, treatment-related adverse effects at any of the sacrifice times.

Colon crypt grades for females were 25 to 92% greater than for males at the 18 and 24 month sacrifices ($p < 0.001$). Both treated and control showed a significant increasing trend ($p < 0.001$) with age. Compared to the 6 month sacrifice, crypt grades increased by 3% by 12 months, 34% by 18 months, and 48% by 24 months.

The rectum showed statistically significant increased cell proliferation in males and females in the 500 and 1000 mg/kg dose groups. Crypt grades for 500 and 1000 mg/kg treated rats at 12, 18, and 24 months were between 3 and 46% greater than in control rats. These changes were statistically significant ($p < 0.0001$) and increased in a dose-related manner ($p < 0.001$). Sex effects were not significant. There was a significant increasing trend ($p < 0.001$) with age in treated and controls. Compared to the 6-month sacrifice, crypt grades increased by 10% by 12 months, 14% by 18 months, and 37% by 24 months.

[However, there was no gastrointestinal histopathologic or neoplastic change after 78 or 104 weeks of dosing.]

Toxicokinetics: Determinations were made from single morning blood samples on a single day at the time of each sacrifice (weeks 26, 52, 78, 104 using 5, 5, 13-15 and 31-36 animals/sex/dose group, respectively). These data and that from the end of 104 weeks of dosing indicate that there was continuous systemic drug exposure throughout the study. In addition, systemic exposure increased over the course of study.

Sponsor's table:

Interim Sacrifice (Weeks)	Plasma Level of Ro 18-0647 (ng/ml; mean \pm SD*)					
	150 mg/kg/day		500 mg/kg/day		1000 mg/kg/day	
	Male	Female	Male	Female	Male	Female
26/27	28.8 \pm	42.0 \pm	153 \pm	144 \pm	246 \pm	849 \pm
	12.2	11.4	47.4	71.5	55.1	407
52	32.1 \pm	42.9 \pm	557 \pm	448 \pm	410 \pm	591 \pm
	17.5	21.3	220	244	215	248
78	51.7 \pm	68.4 \pm	374 \pm	907 \pm	600 \pm	1300 \pm
	36.6	45.2	173	592	332	673

*n=3-5 weeks 26/27 and 52, and 13-15 for week 78

Peak concentrations in week 104 were achieved between 01:00 and 03:00 for male rats and between 05:00 and 07:00 for female rats. Trough concentrations were observed in late morning or early afternoon.

It is reported that consistent with the 52-week toxicokinetic data, the systemic exposure to parent compound increased by 2.4-5.2 fold over the 2-year chronic treatment as expected from the slow rate of elimination of orlistat in rats. For both sexes the systemic exposure was more than dose proportional in the dose range of 150-500 mg/kg and reasonably dose proportional in the dose range of 500-1000 mg/kg. It is reported that no major sex differences were observed, however, the AUC exposure was 20-30% higher in some instances in females.

The sponsor reports that levels of unchanged Ro 18-0647 below 0.2 ng/ml were observed in most of the plasma samples from obese patients treated with 120 mg orlistat t.i.d. for 12 months. Further, that the accumulation of orlistat observed in rat plasma is not relevant for the human situation since all clinical studies indicated a lack of accumulation of the negligibly absorbed orlistat in plasma. The toxicokinetic data are reported to provide a safety factor of above 1000 considering the available clinical data. [A very high dose of 400 mg orlistat t.i.d. (n=6) in humans yielded a maximum plasma concentrations of less than 4, 20 and 50 ng/ml. None of the 12-month plasma samples from obese patients at the clinical dose level of 120 mg t.i.d contained measurable orlistat concentrations i.e. above 0.2ng/ml.]

The concentration of total fecal lipids of treated vs the corresponding control group was increased 3.6, 4.0 and 4.6 fold in males and 3.8, 4.2 and 4.6 fold in females low thru high dose, respectively, showing similarity in both sexes.

APPEARS THIS WAY ON ORIGINAL

One-Year Oral (gelatin capsules) Toxicity Study in Dogs (Protocol No. 122 P 88) Corrected Version of the Original Report B-153'797, dtd 3 Dec 1990 (Research Report B-164'939) - Supplementary data on the composition and concentration of rectal luminal lipids dtd 6 Nov 96. F. Hoffmann-La Roche Ltd and Netherlands Institute for Dairy Research. Pharmacology Review of Original study dtd 16 Jan 92.

Four dogs per group (6 high dose) received 0, 5, 50, 300 mg/kg for 1 year. This portion of the study was to determine the composition and concentration of unabsorbed lipids, and of rectal water parameters considered to reflect potential interactions of lipids with the colonic epithelium. Lipid excretion calculated as percentage of intake was dose-dependent increased, amounting to 21% in control dogs, and 44, 68 and 89% at the dose of 5, 50, and 300 mg/kg/day.

There was an increase in the predominant triglycerides, and a modest increase of diglycerides and monoglycerides, but no change in unesterified fatty acids. According to the sponsor, the lack of increase in fatty acid concentration supports the conclusion that exolipase activity of the microflora previously demonstrated in the same dogs does play either no relevant role in vivo or has been completely suppressed by the lipase inhibitory activity of orlistat at all dose levels tested. They further state that measurements of biochemical parameters of the rectal water phase have not provided any evidence indicative of a detrimental change of the colonic environment or of epithelial cell damage. No significant effect on the concentration of soluble fatty and bile acids, nor on cytolytic activity of rectal water and epithelial release of alkaline phosphatase was observed which are consistent with the absence of any relevant findings upon histological evaluation.

Composition of the Intestinal Microflora of Dogs after Treatment with Different Doses of Orlistat (Tetrahydrolipstatin, THL) for One Year. Report B-156'866 dtd 6 May 93. Study performed at F. Hoffmann-La Roche Ltd. Authors: Isler D., et al. [Ex 1 year study: Lot ?; Batches G PUL: 456 088, 461 088, 463 088, 464 088, 465 089, 467 089, 498 089, 515 089, 516 089, 538 089, 540 089, 543 089, 545 089, 546 089, 547 089, 549 089, 551 089, 556 089.]

Four dogs per group (6 high dose) received 0, 5, 50, 300 mg/kg for 1 year (study during time of the 1-year oral chronic toxicity study in male and female dogs). [According to these authors, on average, excreted lipid accounted for 20, 41, 65, 87% of the dietary intake (control - high dose).] Changes were not provoked in the predominant (the GI tract contains an enormous number and variety of microorganisms) bacterial species of the intestinal flora of dogs. The small dose-dependent increase in Staphylococcal species was not significant. Isolated bacterial and fungal species occurred to the same extent as in controls.

A few of the isolated microorganisms (3 Staphylococcal, 1 Actinobacter species) showed unequivocal lipolytic activity. Reportedly in vitro, the microbial lipolytic activity of the most lipolytic isolate could be potently inhibited by tetrahydrolipstatin.

Special Studies:**The Effect of Orlistat (tetrahydrolipstatin, Ro 18-0647) on Fecal Parameters and In Vivo Colonic Proliferation in Male Wistar Rats:**

Roche Research Report B-156'860. Dtd. Apr 93.

Lot (?). Q.A. - Not Present

Rats were fed a purified high fat/low calcium diet [40% calories as butter fat, 0.1% elemental calcium (17% protein, 43% carbohydrate)] for ca 9 days to model roughly a human Western diet. Relative to the normal rat diet this represents ca 3-fold increased dietary fat and 5-fold reduced calcium. Orlistat was incorporated into the dietary fat. To omit anal leakage of oil, cellulose at a level of 10% had to be added to the diet (orlistat could not be increased above 1.5 mg/g of fat to avoid anal leakage). Rats were treated with dose levels of 0.6 (8.5 mg/kg/day) and 7.5 (117 mg/kg/day) mg/g fat in the 9 day study. The high dose planned was 1.5 mg/g fat (21 mg/kg), but due to error this became 7.5 mg/g fat. Fat absorption was inhibited by ca 24 and 80% at the two dose levels, respectively. Anal leakage at the higher dose level prevented exact quantitation. Thus it appears that orlistat at doses that produce increased fecal excretion of fat may be associated with some increase in colonic diglyceride, monoglyceride and fatty acid concentration. Mucosal cell turnover was increased in a dose-related manner (unchanged total DNA but increased incorporation of thymidine). Unexpected in the current study was reduced fecal output of bile acids and their concentration in fecal water. This study also produced a dose-dependent increase in the excretion of calcium.

10-Day Study of the Effects of Orlistat (Ro 18-0647/008) on Fecal Parameters and Colonic Mucosal Proliferation in Rats Maintained on a Purified High Fat - Low Calcium Diet. Dose-response and reversibility evaluation after 10 days of treatment.

Roche Research Report B-162'806. Dtd Oct 96. Lot 3013013. Diet - The purified diet was based on the composition of the AIN-76 diet except for fat (40% of calories, milk fat/corn oil = 9/1 by weight), calcium (0.1% w/w), and cellulose (10%, w/w) level. [protein 17 energy %; carbohydrate 43 energy %]. Q.A. - Not Present

Groups of 6 male rats each were allocated as follows to the treatment and recovery part of the study: Orlistat treatment (10 days) - 0 (control diet), 0.6, 1.5, 7.5 mg/g of dietary fat; Treatment (10 days) + recovery (10 days) - 0 (control diet), 0.6, 7.5 mg/g of dietary fat. The three dietary levels of orlistat provided doses of ca 8.5, 25, and 127 mg/kg/day in the treatment group and 8.7 and 121 mg/kg/day in the recovery groups. Feces collected daily during the last four days of the treatment or recovery periods were pooled.

The purpose of the present follow-up study of orlistat in rats fed the high fat/low calcium diet was to assess reproducibility, dose-response relationship, and reversibility of the effects observed in the previous study. [To elucidate the underlying mechanism of the proliferative response, fecal diacylglycerol (DAG) and some additional biochemical parameters not previously measured were determined.]

Results included the following: Previously observed stimulatory effects of orlistat on colonic mucosal proliferation were confirmed and a dose-dependency and reversibility were established. 10 days treatment with orlistat produced a dose-dependent increase in colonic mucosal cell turnover (as reflected by increased thymidine incorporation into mucosal DNA) without any evidence of cellular hypertrophy or hyperplasia. Doses of 8.5, 25 and 127 mg/kg/day produced increased thymidine incorporation of 1.9, 2.4 and 5.0 fold along with inhibition

of fat absorption by 29, 58 and >75%. The 127 mg/kg dose produced marked anal leakage of oil which to some extent compromised quantitative fecal lipid parameter assessment. Grooming activity caused fur to be covered all over with oil causing rats to exhibit stress-like excitation. With all dose levels a large fraction of fecal fat was excreted as digestive products including diacylglycerol (DAG) monoglycerides (MAG) and unesterified fatty acid (FFA) which indicated that residual lipolytic activity in the lower intestine was not completely suppressed by the highest dose of orlistat. The current fecal fat excretion may be related to the special composition of the diet, possibly to the milk fat content. Only the high dose impaired net calcium balance which was not significantly altered at the low and mid-doses. However, fecal FFA which can precipitate calcium was markedly increased. None of the doses of orlistat stimulated bile acid excretion. The assumption that at the low and mid doses of orlistat the hyperproliferative response was largely related to epithelial cell damage caused by the cytotoxic activity of increased levels of soluble fatty acids in the colonic lumen is supported by the biochemical fecal parameters measured. Neither fecal fatty acids nor diglycerides adequately account for the more marked increase of proliferative activity at the high dose. There may have been some reaction to stress in connection with grooming and anal oil leakage. The 10 day **recovery period** resulted in colonic epithelial proliferation that was almost comparable to control levels for the low dose, however for the 7.5 mg/kg high dose colonic proliferation was still elevated - thus a longer wash-out period may be needed.

Body weights showed dose-related effects with weight gain recovery during the treatment free period. Compared to controls increased food intake was still evident during the recovery phase.

Three-Week Oral (dietary fat admix) Study of Ro 18-0647/008 (orlistat) in Rats Maintained on Purified High Fat Diets Containing Either Low or High Concentrations of Calcium. Effects on Fecal Parameters and Colonic Mucosal Proliferation:

Roche Research Report B-162'809 dtd. Nov 1996. Lot: 2013009
 Q.A. - Not present. Diet: Based on the composition of the AIN-76 diet except for fat (40% calories, milk fat/corn oil = 9/1 by weight), calcium (either 0.1 or 1.0%, w/w) and cellulose (10%, w/w).

Male SPF-Wistar rats (n=8) aged 9 weeks were fed purified diets ad libitum for 20 days. The diets contained 0.06 or 1.5 mg orlistat per gram of fat (ca 8.5 or 117 mg/kg) and either a low (1 g/kg = 0.1%) or high (10 g/kg = 1%) calcium diet. An additional group of 8 rats consumed powdered laboratory chow.

This study showed the proliferative response of the colonic mucosa in this rat model to be transient. The increase of soluble fatty acids and cytotoxic activity of fecal water was still present after 20-22 days, however, there was no evidence of epithelial damage (release of alkaline phosphatase) and there was no apparent observable effect on colonic mucosal proliferation. Thus, the colonic epithelium is able to adapt (between 10 and 20 days of treatment) to the increased concentration of ionized fatty acids present in the colonic lumen. The mechanism is unknown but according to the sponsor may possibly involve increased production of protective mucus.

This study also showed that the increase of ionized fatty acid concentration and cytotoxic activity of fecal water associated with orlistat

treatment in rats fed a high milk fat, low calcium diet could be abolished by increasing the dietary calcium level (1.0 vs 0.1% w/w). There were clear differences in the profile of soluble fecal surfactants in response to either increased dietary fat or orlistat treatment when comparisons were made of rats fed a purified high fat, low calcium diet with those maintained on a low fat chow diet. The higher dietary fat content was associated with increased concentrations of bile acids, however, orlistat treatment increased only fatty acids (although more markedly); it reduced the soluble fecal bile acid concentration. In addition calcium supplementation abolished the mild oily leakage seen in orlistat treated rats maintained on the high milk fat, low calcium diet. Orlistat retarded growth - most clear in calcium supplemented groups, however weight gain of the 0.6 mg orlistat/g of fat and low-calcium was slightly higher than that of the control group with no orlistat and low-calcium.

Thus, it appears that calcium phosphate supplementation during orlistat administration does not interfere with the intended effect of orlistat on dietary fat absorption and growth, but prevents the orlistat-induced detrimental changes in colonic physiology.

Four-Week Oral (feed admix) Toxicity Study (Dose-ranging) in Han lbm:WIST (SPF)

Rats Fed a High-fat/Low-calcium diet: F. Hoffmann-La Roche Ltd., Switzerland. Research Report B-164'932 dtd Aug 96. Protocol 108 P 95. Batch 3084028 Code No. Ro 18-0647/V01-00 (formulated material Lot G QC 0080/02) [1% premixture of Ro 18-0647/008 = lot 301439] Q.A. - Present

The object of this dose range-finding study was to supply basic data for the planned long-term study.

Diet - Kliba 10-343 Sp2 - contains 18.4% (w/w) lipids (40% calories as fat - mainly in the form of pork fat) and 0.12% (w/w) calcium.

Male and female Wistar-stock rats were assigned to groups of 10/sex (groups A, B, C, D, E) or 4/sex (group F). Food and water were given ad libitum.

From weeks 2 to 4 Ro 18-0647/008 was administered orally by feed admix at daily doses of 0 (A), 70 (B), 140 (C), 280 (D) and 560 (E) ppm (0.4, 0.8, 1.6, 3.2 mg of Ro 18-0647/008 per gram lipid relating to one kg rat diet). Four rats/sex (F) treated with 560 ppm were used solely for toxicokinetic study. Group A controls received powdered diet without drug. Calculated mean dose levels (mg/kg) weeks 2-4 were: Gp B 3.14 - 4.23; Gp C 6.86 - 11.35; Gp D 16.31-24.90; Gp E 33.37 - 57.72. [Due to a dosing error during week 1, rats were treated with 10% of dose levels given during weeks 2 to 4.]

There were no mortalities. No drug-related findings were reported for body-weight or organ weight changes (however, kidney weights were slightly increased in Gp D and E males and Gp E females), or for hematology - various statistically significant differences reported as not biologically meaningful), gross and histopathology (findings considered background).

Drug related findings included: Greasy coat of both sexes of the two higher dose groups; Increased feed intake for both sexes at 140 ppm and up; Serum urea increased (possibly from increasing protein absorption with increasing dose levels) for both sexes (M by 17 to 29%; F by 2 to 37%) of all dose groups;

Increased fecal lipid excretion for both sexes of all dose groups [% of lipid intake - Gp A ♂3.2, ♀2.9; Gp B ♂20.8, ♀14.8; Gp C ♂45.4, ♀49.4; Gp D ♂66.8, ♀70.2; Gp E ♂75.6, ♀70.0]. The concentration of lipid in dried feces

increased up to 40 (males) and 46-fold (females) relative to control levels of untreated rats. Total feed intake, weeks 1-4, increased by 9 to 61% in both sexes of the three higher dose groups - probably compensation for calorie deficit.

Toxicokinetic monitoring showed only sporadically measurable concentrations of unchanged drug above the limit of quantification (5 ng/ml) [not unexpected from low doses administered and low GI absorption/extensive first-pass metabolism in the rat].

Supplementary Data re Research Report B 164'932 (above): [Protocol 108P95] Roche Research Report B-162'807 dtd 15 Oct 96.

Tri- and diglyceride excretion increased with increasing dose, however, a biphasic response of daily excretion of unesterified fatty acid was observed. The largest amount of fatty acids excreted was at the 140 ppm dose (8.9 and 11 mg/kg in males and females); it was less at higher dosages. In relative terms excretion of fatty acid at 70 ppm was 3 to 4 fold larger than that of triglycerides, however, at the 140 ppm level the relation was reversed and 1.5 to 2 fold more triglyceride was excreted. The two lower doses showed ca 15% of the fatty acid was excreted as 10-hydroxy derivatives. 10-hydroxy fatty acids were not detectable in control rat feces and only negligible amounts were found in the fecal lipids of rats treated with the larger 280 and 560 ppm doses. [It is reported that the fecal hydroxy FA which were excreted in response to the lower dose levels of orlistat, probably originate from microbial metabolism. **Males only**, showed a dose-dependent reduction in the daily excretion of bile acids. [Control males showed larger daily BA excretion than female controls.]

The fecal concentration of diglycerides was increased ca 10-12 fold at 140 ppm, and ca 25-30 fold at 280 and 560 ppm. Control values were below the limit of quantitation. For the fecal water phase the concentration of soluble fatty acids increased 6.6, 10.8, 2.8 and 1.9 fold for males and 4.9, 6.1, 2.3, and 1.3 fold for females for the low dose through the high dose, respectively. The soluble bile acid concentration decreased with the dose by a maximum of ca 50%.

Supplementary Data re Research Report B 164'932 (above): [Protocol 108P95] Roche Research Report N-138796 dtd May 1996. Nutley Study 06800. Q.A. - Present

Sections of colon were stained for Proliferating Cell Nuclear Antigen (PCNA) and BrdU by immunohistochemistry and evaluated by labeling index and crypt grade for cell proliferation.

The increase in cell proliferation observed in the colons of males treated with 140, 280 and 560 ppm Ro 18-0647/008 was proportional to dose. Evidence of increased cell proliferation was detected in females at doses of 280 and 560 ppm.

Although generally consistent, in males staining for PCNA detected a proliferative effect at a lower dose (140 ppm) compared with BrdU staining (280 ppm). Staining in females was less consistent than in males. BrdU staining for females gave a significant decrease in the labeling index for 70, 140 and 280 ppm rats compared to controls in which the labeling index was abnormally high.

APPEARS THIS WAY ON ORIGINAL