decreased plasma levels. These were not fully compensated for by dietary vitamin supplementation. In dogs, plasma cholesterol was decreased. Plasma Vitamin D was also decreased. Other findings were similar to rats. Since most of the Orlistat is not absorbed, effects on the g.i. tract were of particular interest. This was studied in a number of models in diets with high fat and low or normal calcium. No g.i. neoplasia or hyperplasia was observed. However, a reversible increase in colonic proliferation was observed. In rats, there was a treatment-related increase in the number of colonic aberrant crypt foci in rats on a high fat normal calcium diet for 9 months. The significance of this finding is unknown and has not been detected in humans.

5. **Mutagenicity**: There were no findings of mutagenic potential for Orlistat in the Ames bacterial mutagenesis assay, V79/HGPRT mammalian mutagenesis assay, an in vitro clastogenicity assay in peripheral human lymphocytes, an in vivo mouse micronucleus assay or UDS assay.

6. **Carcinogenicity**: Rat studies performed at doses >29 times the human exposure (based on surface area) did not result in any carcinogenic potential. Mouse studies performed at exposures >22 times the human exposure (based on surface area) resulted in a statistically significant finding ($p = 0.0248$)of liver hemangiosarcomas in high dose females. This finding was significant due to the rarity of the tumor in this strain and amounted to a finding of two tumors in the high dose female group while there were no tumors in any other group. When the statistical analysis was repeated combining the incidences of hemangiomas and hemangiosarcomas, the finding was not statistically significant. There were no statistically significant findings of tumors in the g.i. tract.

7. **Reproductive Toxicology**: Absorption in rabbits was sporadic and variable, therefore, exposure comparisons for the reproductive studies will be based on mg/m2. Exposures were 23 and 47 times the clinical dose for high dose tested inrat and rabbit, respectively. There were no teratogenic findings in rabbits. In one rat study, there was an increase in dilated cerebral vesicles in pups from the mid and high dose groups (6 and 23 times human exposure, statistically significant in the high dose group). However, this was not repeated in two other rat studies.

APPEARS THIS WAY ON ORIGINAL

XENICAL (orlistat) [Ro 18-0647; tetrahydrolipstatin; THL] is a hydrogenated derivative of the pancreatic lipase inhibitor Lipstatin. It is a long-acting lipase inhibitor indicated for the treatment of obesity. In contrast to currently available antiobesity drugs which depend on appetite suppression, orlistat's therapeutic activity is exerted in the lumen of the stomach and small intestine by forming a covalent bond with the active serine site of gastric and pancreatic lipases. The enzymes, thus inactivated, are unavailable to hydrolyze dietary fat in the form of triglycerides into free fatty acids and monoglycerides for absorption. Since the undigested triglycerides are not absorbed, there is a caloric deficit which is the cause of the weight loss. Orlistat is not selective for gastrointestinal lipases but inhibits a broad range of physiological tri- and diacylglycerol lipases including gastric, pancreatic, carboxyester, lipoprotein, hepatic, and hormone-sensitive lipases. However, systemic absorption of the drug which is negligible clinically is not needed for activity since the caloric deficit has a positive effect on weight control. There are no apparent effects on the CNS, cardiovascular system , or immune system that would affect safety.

The interaction with lipases is highly specific and thus orlistat would be unlikely to interfere to any significant extent with the digestion and absorption of proteins and carbohydrates. Re-activation of lipase is possible, but according to the sponsor, far too slow to have any significant pharmacological relevance in vivo.

Fat absorption is inhibited by orlistat in lean and obese rodents as well as in dogs, monkeys, and hamsters with an absence of tolerance or cumulative effects. The effect on fat absorption is reversed after withdrawal of orlistat. Cholesterol absorption is also inhibited by sequestration in unabsorbed fat in the intestinal lumen. There does not appear to be any important effect on digestive and absorptive steps following lipolysis. Dose-dependent antiobesity effects are seen in animal models of obesity. Some hypolipidemic properties are also present.

Orlistat unfortunately has some undesirable effects on gastrointestinal absorptive functions which include altered absorption of lipid-soluble vitamins, ß-carotene, and ω3-fatty acid-rich triglycerides. Orlistat reduced absorption of apolar lipids such as, cholesterol, the lipid-soluble vitamins A, E, and D, as well as ß-carotene, but had no effect on vitamin K function (prothrombin time). Vitamin E (α-tocopherol) and ß-carotene were more markedly and more immediately affected than vitamin A.

Although the fecal concentration of potentially toxic unesterified fatty acids and diglycerides is increased by orlistat, the level of bile acids is decreased. [Bile acids are increased in response to increased dietary fat alone.]

Short-term studies were conducted in which rats were fed a high fat-low calcium diet. Orlistat treatment for 9 or 10 days was associated with a dose-dependent reproducible and reversible increase of colonic mucosal proliferation along with inhibition of fat absorption.

Standard rat chow is relatively high in dietary calcium which is known to lower the concentration of soluble unesterified fatty acids in the colonic lumen. The typical human Western diet is relatively high in fat but low in calcium.

Because of the increase in the rate of cell turnover in the colonic crypts observed in rats fed a high fat-low calcium diet following short term administration of orlistat and its implication in humans, HFD-510 Pharmacology requested the sponsor to conduct a longer term study. Nine month rat studies utilizing a high fat-low calcium diet and a high fat-normal calcium diet were agreed upon.

The high fat-low calcium study (to mimic the "Western-style" diet) was carried out at doses of 0, 70, 140, 280 ppm Ro 18-0647/008. 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. At the 26 week sacrifice, mean crypt heights (number of epithelial cells per crypt column) were significantly greater for high dose males compared to controls. Mean crypt grades for some intestinal sections showed some differences which were still significantly greater than controls after the recovery period at 34 weeks. The incidence of aberrant crypt foci (ACF) at the 26 week period was not considered different by the sponsor and no differences were noted in the incidence of ACF at the 34 week recovery period. According to the sponsor, no treatment-related findings were reported in intestinal parameters evaluated; there were no treatment-related changes in incidence or severity of aberrant crypt foci (ACF) or intestinal histopathological changes. At the final 39 week sacrifice there was, however, a treatment-related increase in crypt height for the high dose females compared to controls. There appeared to be a mild trend in improvement of ACF parameters at the 39 week sacrifice (See Table p. 29).

In general the sponsor reported that because of the small percentage change and lack of dose proportionality, there were no treatment-related differences in changes in the incidence of severity of aberrant crypt foci or histopathological changes or increase in colonic cell proliferation.

The high fat-normal calcium study in rats was also carried out with doses of 0, 70, 140, 280 ppm Ro 18-0647/008 (ca 2.8, 6.7 and 15.3 mg/kg/day for males and 4.4, 10.0 and 22 mg/kg/day for females). It was reported that there were no differences in mean labeling index, crypt height, or crypt grades considered to be treatment-related. There were some significant findings, however, which according to the sponsor were in general a small percentage change with a lack of dose proportionality.

The incidence of aberrant crypt foci were reported to show no difference considered to be treatment-related at the 26 week period. Compared to controls at the end of the recovery period (at 34 weeks), there was a dose-related decrease for males and a dose-related increase for females.

At the final sacrifice, however, there was a treatment-related increase in the number of colonic aberrant crypt foci noted in mid- and high dose females. This number was still increased for females after the recovery period. 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. Male rats did not show comparable effects and there was no increase in ACF in the high fat/normal calcium parallel study in which the dietary calcium levels was 10-fold less. Since Ro 18-0647/008 is not genotoxic or carcinogenic in rats at doses considerably greater, the clinical relevance of this finding is uncertain.

It is reported that measurements of biochemical parameters of the rectal water phase in the year dog study 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 (indicator of cell damage) was observed which are consistent with the absence of any relevant findings upon histological evaluation.

An Expert Committee was approached by the sponsor regarding colonic mucosal hyperproliferation and the possibility of colon cancer. The panel concluded that overall the data available (preclinical and clinical) did not raise significant concerns regarding the effect of orlistat on colonic mucosal hyperproliferation. [The results from the 9 month high fat-low calcium and high fat-normal calcium rat studies were not in at that time.]

Colonic effects have been investigated in humans.

In two other rodent obesity models, diet-induced obese rats treated subchronically with orlistat which inhibited fat absorption by at least 60% showed a reversible increase in weight of the large intestine. There were no appreciable histopathological findings. The sponsor attributed the increase in tissue weight to a normal physiological adaptive response to increased amounts of unabsorbed bulk of fat in the intestinal lumen.

Under fed conditions, alterations (reversible) in plasma triglyceride metabolism in rodents and dogs were seen only at doses which exceeded the clinically recommended dose. There was considerable variation between species in threshold levels of the oral dose and resulting plasma levels of orlistat producing a measurable increase in plasma triglycerides. Such findings were affected by diet composition and mode of drug administration. However, the fatty changes and fatty infiltrations of the adrenal cortex, liver, and bone marrow, seen in rats treated at doses $\geq$500 mg/kg/day in the chronic studies are probably attributed to inhibition of cellular lipases such as lysosomal lipase in animals.

The role of lipases in the digestion and absorption of fat and in systemic lipid metabolism is very similar across mammalian species. Studies of lipases in vitro and measurements of fat absorption have shown that the inhibitory potency of orlistat differs only minimally between homologous enzymes of different species and between the different animal models used for study.

Orlistat possesses 4 centers of chirality; thus, there are 16 possible diastereoisomers. Among the other 15 stereoisomers, one exhibited an activity ca 2.5x lower than that of orlistat, with all other stereoisomers at least 8 times less active. Most of them were ca 1000x less active or inactive.

Opening the ß-lactone ring leads to a drastic loss of inhibitory activity although a slight residual activity may be retained due to affinity of these compounds to the substrate binding site of the lipase.

Orlistat does not interfere with triglyceride absorption at digestive and absorptive stages that follow lipolysis, i.e. micellar solubilization of fatty acids and monoglycerides, absorption at the brush border membrane, re-esterification in the enterocytes, incorporation of re-formed triglycerides into lipoproteins, and secretion into the lymphatics.

It has been shown in rats (3-week study) that the effect on ionized fatty acid concentration and cytotoxic activity of fecal water associated with orlistat treatment (~8-9 and ~24-25 mg/kg) of rats fed a diet high in milk fat and low in calcium was abolished by increasing the dietary calcium level (1.0 vs 0.1%). Thus, from this study it would appear that orlistat is unlikely to increase soluble fatty acids and colonic mucosal proliferation in rats as long as a high-calcium diet is provided. However, the 9 month study with high fat-normal calcium (for rat feed) produced a treatment related increase in the number of colonic aberrant crypt foci in mid and high dose (10 and 22 mg/kg) females. The significance of this finding is uncertain. There were no findings of g.i. tract tumors in the rat or mouse 2-year carcinogenicity bioassays performed with normal calcium levels.

It is reported that a clinical study in obese patients showed that no colonic epithelial cell proliferation was apparent after treatment with orlistat for 6 weeks.

A reproducible, dose-dependent increase in the wet weight of the large intestine and, to a lesser extent, of the small intestine is caused by orlistat in diet-induced obese rats. Fat absorption was inhibited by at least 60%. The effect on tissue weight was reversible after discontinuation of treatment, and was not associated with any histopathological findings. However, orlistat treatment of genetically obese mice and rats had no significant effect on the weight of the intestine, although the highest dose induced similar fractional inhibition of fat absorption to that observed in diet-induced obese rats.

At moderate inhibition of triglyceride absorption, a significant fraction of fat is excreted as unesterified fatty acids. The luminal availability and absorption of certain minerals might be affected such as calcium, magnesium and zinc which can combine with unabsorbed fatty acids to form insoluble soaps. Rats fed a high-fat diet at calcium levels slightly below the corresponding average intake in humans were reported to have only a very weak reduction of net calcium absorption in spite of a marked increase in the excretion of unesterified fatty acids.

Long-term high exposure to unabsorbed fat in orlistat studies in dogs did not show any significant effect on the composition of intestinal microflora. The dose-dependent increase in daily fecal lipid excretion was rather constant throughout the 1-year treatment period. The amount of lipid excreted at the

highest dose (300 mg/kg/day) was 6-fold greater than that in control animals. The concentration of free fatty acids in the rectum was not increased by treatment, but showed a dose-dependent reduction.

Gastric emptying did not appear to be significantly affected by orlistat. Regardless of the dose used, orlistat given with mixed meals had no physiologically significant effect on either the rate of gastric emptying in rats or on the overall gastrointestinal transit time of normal indigestible bulk of meals in rodents and dogs.

Neither the toxicological studies in rodents and dogs nor subchronic evaluation in obese rodents showed any indication of a functional exocrine pancreas hypertrophy.

Short-term studies conducted in obese mice and rats support the conclusion that orlistat has no direct or indirect effects on the overall process of bile acid production and biliary secretion of bile acids and cholesterol other than those attributed to the reducing effect on lipid absorption and/or the presence of unabsorbed lipid in the intestine.

Studies in rats and dogs suggest that the threshold plasma level of orlistat above which triglycerides may increase, differs between species (rat vs dog) and is influenced by the mode of administration (diet admix of the pure vs the pelleted drug in rats). However, according to the sponsor in vitro data suggest that at a given concentration of orlistat in lipoproteins, the inhibitory effect on lipoprotein lipase and thus the effect on plasma triglycerides should be rather uniform.

Total absorption of [redacted]C-labeled orlistat was relatively low and variable in rats and dogs ranging from 1 to 22% under physiological conditions. The extent of absorption depended on the formulation as well as the dietary status of the animals. In general absorption of drug-related material was not markedly affected by the dose. Mid-meal administration of oral doses produced a significantly higher absorption than that produced during a fasted state.

Following oral administration of 1000 mg/kg in short term toxicity studies the $AUC_{(0-24h)}$ ng•h/ml in males/females was 489/281 for mice, 7690/12200 for rats, and 21100/16000 for dogs. For female rabbits (males not tested) following 800 mg/kg orally, the value was $\leq$30. Plasma concentrations were barely quantifiable in rabbits despite the high dose administered for 13 days.

Single dose i.v. pharmacokinetics in both dogs and rats showed a low systemic plasma clearance of 12-13 ml/min/kg. The liver extraction ratio for the isolated perfused rat liver was 0.24. Consistent with the high lipophilicity of the drug, the volume of distribution (114 l/kg) and plasma half-life (130 h) were large in the dog indicating extensive tissue penetration. Values were not obtained for the rat in this study. Multiple dose kinetics were assessed in short and long term studies in mouse, rat and dog. For rodents the plasma concentrations of orlistat tended to increase during chronic administration, possibly due to a slow elimination rate. However, orlistat did not accumulate in dogs during chronic oral administration. Actually there was a decrease in exposure by the end of the year, possibly due to a decrease in absorption. In general systemic exposure was more than dose proportional in female mice, rats and male dogs. In some cases gender differences in systemic exposure were noted i.e. higher systemic exposure in male than female mice. However, in most cases it was difficult to assess such effects due to the large intra- and inter-animal variability.

In vitro orlistat was >99% bound to plasma proteins (lipoproteins and albumin were the major binding proteins). Orlistat was minimally portioned into erythrocytes.

Based on in vitro data, it appears that the metabolism of orlistat occurs mainly within the gastrointestinal wall. The small fraction of the dose that is absorbed undergoes extensive first-pass metabolism, primarily during the transfer through the gut wall and to a lesser extent in the liver. Thus very little intact orlistat (<1%) of the dose) reaches the systemic circulation. Hydrolyzing enzymes in plasma produce further degradation.

Two main metabolites seen clinically, M1 (4-membered lactone ring hydrolyzed) and M3 (M1 with n-formyl leucine moiety cleaved), account for ca 42% of total radioactivity in plasma. Further metabolism produces a shortening of the $C_{11}H_{23}$ side-chain and conjugation with glucuronic acid leads to a number of more polar secondary and tertiary metabolite products. All of the metabolites thus identified have a hydrolyzed ß-lactone ring and are thus devoid of relevant lipase inhibitor activity. M1 and M3 have an open ß-lactone ring and reportedly extremely weak systemic lipase inhibitory activity (1000- and 2500-fold less than orlistat, respectively). The inhibitory activity is low and plasma levels are low clinically. The metabolites of orlistat have no lipase-inhibiting activity which is likely to be relevant in vivo and thus probably do not contribute to any significant extent to effects seen in animals. Qualitatively the same metabolites are found in the plasma and urine of rats, dogs and humans, suggesting that orlistat is metabolized by the same pathway in these species.

M1 and M3 are the main metabolites in human plasma, while M9 (Ro 61-0591) is the main metabolite in rat and dog plasma after oral orlistat administration. Toxicity studies showed that exposure of mice rats and dogs to these metabolites increased dose proportionally or more than dose proportionally in parallel with intact drug. When metabolite levels were compared with those of intact drug in mice, the metabolite levels largely exceeded those of orlistat, while the orlistat and metabolite levels were similar in rats, and higher orlistat than metabolite levels were observed in dogs.

The only metabolite of orlistat potentially able to inhibit lipases on the basis of its intact ß-lactone ring is the putative metabolite, Ro 40-1379 which however, is not detected in plasma or tissues of animals after high doses of orlistat.

Hepatic metabolism is reported to contribute little to the formation of primary metabolites. In a single-pass experiment in an isolated perfused rat liver most (86%) of the radioactivity remained in the perfusate and 80% of this was unchanged orlistat. The extraction ratio under these conditions is only 0.24.

Excretion of drug-related material following ^{14}C-labeled orlistat was essentially complete after four days in rats and after 7 days in dogs. Most of the radioactivity was excreted in the feces with only up to 11% being recovered in the urine in both species. $^{14}CO_2$ contributed very little to overall excretion.

Interspecies comparisons show oral absorption to be low in all species studied (mice, rats, rabbits, dogs, man). In general protein binding and metabolic pathways are similar in these animal species and man. In mouse, rat, rabbit and dog the systemic availability of intact orlistat is generally <1%. The systemic availability of orlistat is reported to be negligible in humans

because following oral administration of therapeutic doses plasma levels (<10 ng/ml) are extremely low. The high oral doses used in the chronic toxicity studies produced a moderate accumulation of orlistat in plasma i.e. for mice 2-3 fold, and for rats 3-5 fold, however, for dogs there was a 4-5 fold decrease of exposure. Reportedly humans have shown no clear trends towards either an increase or decrease in plasma orlistat levels.

The exposure of animals (mouse, rat and dog) to orlistat and its main metabolites at the high doses used in toxicology studies was in most cases considerably greater than levels in humans under therapeutic conditions. Orlistat C_{max} values of 2000 to 3000 ng/ml were seen following 1000 mg/kg doses during the 2-year carcinogenicity study in rats and the 1-year dog study with no serious toxicities. The maximum concentrations of metabolites M1 and M3 in mice and rats are reported to exceed levels in humans by a factor of 3 to more than 10.

Orlistat pharmacokinetics showed single oral doses to have a negligible systemic availability in animals i.e. $\leq$0.1% in rat, and 0.7% in dog. This is probably due to low GI absorption and an extensive intestinal first-pass metabolism. After reaching the systemic circulation, orlistat distributes extensively into organs and tissues (volume of distribution in dogs is 114 ± 84 l/kg). It is cleared slowly. Plasma clearance in rats and dogs is 12-13 ml/min/kg with a terminal $t_{½}$ in dogs of 130 h.

When large radioactive doses were given to rats for 30 days, orlistat-related material was largely confined to the GI lumen, with low levels in tissues including the intestinal walls. For other tissues the liver, kidney and white and brown fat showed the highest concentrations of radioactivity.

Toxicity Studies:

Acute toxicity studies showed no lethality at doses up to 5000 mg/kg in rodents (including 2-week old rats) and 1000 mg/kg in dogs. [No apparent drug-related mortality or adverse clinical signs were observed in mice and rats at i.v. doses of 100 or 150 mg/kg.

Multiple Oral Dose Studies in Mice:

A 13-week oral (dietary admixture - [redacted] polymorph B) toxicity study was conducted in mice at doses of 0, 10, 65, 400, 2500 mg/kg/day. This study served as a dose range-finding study for the carcinogenicity study.

There was no unscheduled mortality or clinical signs of intolerance. Body weight gain was not affected but food consumption was increased in high dose males and for females at all dose levels. Plasma total cholesterol was slightly decreased and plasma triglyceride levels were slightly increased at the three higher dose levels. There were no apparent drug-related organ weight changes, gross or histopathological findings. Dose-related decreases were seen for hepatic concentrations of vitamin A (ca 30-60%) and vitamin E (ca 7-40%).

The 13-week oral study in mice did not provide sufficient information for selecting carcinogenicity study doses. Thus, a 2-week oral (dietary admix) study was conducted at nominal doses of 0, 50, 500, 1000, 2500 mg/kg and systemic exposure was determined. Data indicated that a nominal dose of 1500 mg/kg/day should provide an AUC_{0-24h} of at least 30 fold greater than that associated with the clinically effective dose of orlistat.

The **mouse carcinogenicity study** was carried-out orally at doses of 0, 0, 25, 375, 750 and 1500 mg/kg/day with vitamin supplementation. The three highest doses were selected with the agreement of the Division (HFD-510). The end-of-study toxicokinetic analysis gave an AUC value for the high dose of orlistat

(1500 mg/kg) which was approximately 70 times the AUC for humans. The AUC in humans for the 120 mg t.i.d. dose, 13 ng.h/ml was derived by linear extrapolation of the data from an earlier clinical trial conducted at a dose of 400 mg/kg for 10 days. Assessment of systemic exposure in animals to unchanged Ro 18-0647/008 at various periods showed no unchanged drug at the low dose. Other groups showed dose-related increases in plasma Ro 18-0647. Levels in males were 1.5 to 2-fold higher than in females. Systemic exposure showed approximately a 1.3 to 2.8-fold increase over the course of study. Other findings included: increased food consumption without a concomitant effect on body weight gain (body weight gain of high dose male mice was slightly lower than that of controls); despite vitamin supplementation hepatic vitamin E levels were decreased in the 3 highest dose groups.

Mild increases in colonic cell proliferation tended to increase with dose and duration of exposure to drug. 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.

Composition of fecal lipid for the most part showed little change from control with respect to fatty acid, diglyceride, and triglyceride excretion. The low efficacy of Ro 18-0647 observed in this study relative to the inhibitory effect on fat absorption is unexplained.

In general the nature and incidence of neoplastic and non-neoplastic lesions were similar to that of controls. However, liver hemangiosarcomas (2) in females were seen only in the high dose group. According to analysis by FDA (HFD-715), the positive linear dose-response trend for liver hemangiosarcoma in female mice indicated that this trend was statistically significant ($p=0.0248$). A cut-off p-value of 0.025 was used (a criterion set by the Agency), based on the following facts: 1) there were no occurrences of this tumor in the control groups (i.e., 0% of spontaneous incidence rate), 2) this tumor was identified as a fatal tumor.

HFD-715 was asked by the reviewer to do an analysis of dose-response trend in the mouse for hemangioma-hemangiosarcoma combined and separately. These tumors were analyzed separately and together as a single tumor type. The analyses showed no positive linear dose-response trend in the mice (male and female) for the above tumors.

According to survival data analysis (produced by HFD-715), the positive dose-mortality trend was not statistically significant in either sex in mice.

Multiple Oral Dose Toxicity Studies in Rats:

Multiple dose oral (dietary admix) toxicity studies were conducted in rats for 13 weeks at doses up to 450 mg/kg (crystalline polymorph A) and 2500 mg/kg/day (fine milled, polymorph B) and at doses up to 125 mg/kg/day for 52 weeks. Vitamins (A, D_3, E, and K) were supplemented for the 13-week high dose study and the carcinogenicity study. The higher dose 13-week study provided data for the carcinogenicity study which was conducted at doses up to 1000 mg/kg/day for 104 weeks.

Findings in the rat studies were in general consistent and dose related. There did not appear to be any treatment-related mortality or treatment-related clinical signs other than evidence of increased fecal fat excretion resulting in loose/soft stool, unkemptness and oily fur. At doses of $\geq$125-150 mg/kg/day effects included increased food intake without concomitant increases in body weight gain, hypertriglyceridemia, hyperbilirubinemia, increased plasma cholesterol (at doses $\geq$450 mg/kg/day), increased plasma amylase activity, increased BUN, decreased liver concentrations of vitamins A and E (sometimes accompanied by plasma level decreases) and increased fecal fat excretion. Doses

≥1000 mg/kg/day showed histopathological changes consisting of fatty infiltration and fatty change especially in the adrenal cortex, and sometimes in kidney, bone marrow and the lumen of heart vessels. In general orlistat was not detectable in rat plasma at oral doses <125 mg/kg/day.

The decrease in mean serum amylase activity and increase in mean BUN levels were more pronounced for the high-dose groups and were probably adaptive metabolic changes associated with the alteration in dietary intake of fat and protein secondary to the dose-related inhibition of fat absorption, pharmacological effect, and relatively high protein diet consumed (decreased fat absorption leading to increased food intake required to maintain daily calorie intake). [Laboratory rodents are known to adjust their food consumption to maintain energy intake if the caloric density of the diet is reduced.] It is reported that a high protein diet has been previously associated with decreased plasma amylase.

An oral (dietary admix) **rat carcinogenicity study** of orlistat (fine-milled, polymorph B) was conducted at doses of 0, 0, 150, 500, and 1000 mg/kg/day [determined in agreement with FDA (HFD-510)] with weekly vitamin supplementation (vitamins A, D_3, E, K and ß-Carotene) and interim sacrifices. Selection of the high dose of 1000 mg/kg/day was based on plasma levels of orlistat achieved during a 13-week oral toxicity study and clinical plasma levels from a study at 400 mg t.i.d. for 10 days. The high dose was supposed to provide a systemic exposure to the rat ca 100 fold greater than that achieved clinically by 400 mg t.i.d. At the end of the study, the systemic exposure at the high dose in rats was reported to be about 1000 fold greater than the systemic exposure at the clinically effective dose of 120 mg t.i.d as determined in phase III clinical trials.

It is reported that survival and clinical signs of intolerance did not differentiate treated from controls, however, there was a higher percentage of deaths in the high dose group than in the other groups. Within the first week food consumption was increased in all dose groups. Body weight decreases were seen later in the study. Male and female rats sacrificed at 26, 52, and 78 weeks showed increased adrenal weights due to diffuse cortical hypertrophy at the two higher dose levels. The two higher dose groups also showed an increase in turnover of circulating erythrocytes with a mild reticulocyte response. This effect might have been secondary to lipemia in these groups which could have rendered the erythrocytes susceptible to hemolysis. It is also reported that decreased plasma vitamin E could have resulted in an increase in the lipid peroxidation of the erythrocyte membrane.

Hepatic vitamin A levels were slightly decreased in treated males but not in treated females. Even with vitamin supplementation, liver vitamin E levels were moderately to markedly reduced (males>females) in all treated groups.

A variety of clinical pathology parameters seen primarily in the two higher doses were of the type mentioned in the general statements above and overall the toxicological profile was similar to that of shorter duration rat studies.

Colonic/rectal cell turnover (using PCNA immunohistopathology) assessed after 26, 52, 78 and 104 weeks was not affected by treatment. A minimal increase in cell proliferation in the rectum was observed after 52 and 78 weeks.

Systemic exposure was noted at all dose levels at all time points. Systemic exposure was more than dose proportional between 150 and 500 mg/kg and dose-proportional between 500 and 1000 mg/kg/day. Exposure increased over the 104 week dosing period 2.4 to 5.2 fold which was consistent with the previously noted slow elimination rate in the rat. In some instances female plasma levels exceeded that of males. [The drug does not appear to accumulate clinically.]

No apparent orlistat-related neoplasia was evident.

According to survival data analysis (performed by HFD-715), a positive dose-mortality trend was statistically significant in male rats. This may be explained by the substantially higher percentage of death in the high (1000 mg/kg/day) dose group than in the other groups. The same trend was not statistically significant in female rats.

Multiple Oral Dose Toxicity studies in Dogs:

Multiple dose oral toxicity studies were conducted in dogs. They include 13 and 52 week studies with doses up to 300 mg/kg/day. A second 52-week study at doses up to 1000 mg/kg/day was conducted in which ca 40% of the daily caloric intake was provided as dietary fat (normal calcium).

The 13-week study showed no orlistat adverse effects. Although no toxicokinetic assessment was possible, the data did show bioavailability in the dog.

Effects in the 52-week study were similar to those for rats. The main dose-related effects included increased food consumption (~20-65% for males and ~20-55% for females) without a resulting increase in body weight, decreased plasma cholesterol (~18-57% for males and ~13-36% for females), decreased plasma levels of vitamins D_3 (not noted in rats) and E, and increased excretion of fecal fat (34-90%). Hepatic vitamin A and E concentrations were also decreased. Necropsy revealed no adverse gross pathology and histopathology was unremarkable. Plasma levels were quite varied. Plasma samples showed no unchanged orlistat for the low dose (5 mg/kg). Plasma values ranged up to 620 ng/ml for the mid-dose (50 mg/kg) and up to 1880 ng/ml for the high dose (300 mg/kg).

No apparent adverse histopathological lesions were noted in dogs fed a high fat, normal calcium diet with doses of orlistat up to 1000 mg/kg/day for 1-year. There was no unscheduled mortality nor clinical signs of intolerance. However, food consumption was increased 48.9 - 103.2% (not dose related and F>M), plasma urea was increased 33-63% (M>F)and decreases were seen in plasma cholesterol 49-73%, plasma vitamins D and E 42-83%, and hepatic vitamins A and E 49-88%. Postprandial hypertriglyceridemia was present in mid and high dose groups. Both orlistat and metabolite M1 (Ro 42-3988) were detectable in plasma from the mid and high dose groups throughout the study.

Multiple Intravenous Dose Toxicity Studies in Rats and Dogs:

Since the indicated route for orlistat is oral, the intravenous studies will receive only a general mention here in order to give an indication of the type of toxicity that could conceivably be present following systemic absorption of orally administered orlistat. It was stated that the objective of intravenous studies was to assess and evaluate the toxicity and potential risk of Ro 18-0647/053 (mixed micellar solution formulation) which was intended for the use as an intravenous formulation in human metabolism and pharmacokinetic studies.

Three two-week i.v. studies were conducted in rats with 3 different formulations at doses not exceeding 100 mg/kg/day. Results were somewhat similar from study to study except that local tolerance at the injection site varied with the formulation. In general toxicity seen was often qualitatively similar to that seen with oral dosing. However, reflecting the difference in the route of administration the incidence and/or intensity of intolerance was frequently greater than that observed with higher oral doses. Fatty changes were seen in the liver and spleen in most treated. Single cell hepatic necrosis was also present in a number of high dose rats.

Two-week intravenous multiple dose studies were conducted in dogs with different formulations of orlistat up to 125 mg/kg/day. Dose related adverse effects included postprandial hypertriglyceridemia, marked hepatic toxicity and clinical pathological alterations such as increased plasma transaminase activity, hyperbilirubinemia, increased plasma alkaline phosphatase activity and hypocholesterolemia. The high dose produced yellow-colored livers, moderate multifocal hepatitis with marked fatty change. High dose females lost ca 10% body weight. Findings at 25 mg/kg were mainly confined to an increase in ALAT and marginal postprandial hypertriglyceridemia. The t½ was ca 4.7 hr. Clearance rates (ml/min/kg), volume of distribution (L/kg) and AUC (mcg•h/ml),respectively for the 25 mg/kg dose were 12, 6, and 39 and for the 125 mg/kg dose 8, 4, and 303. [The first sample was obtained after 1 hr.; thus, systemic AUC and t½ might be underestimated.]

Reproduction Studies:

Segment I, II and III reproductive studies with orlistat were conducted as appropriate in rats and rabbits. Oral doses (gavage) ranged up to 400 mg/kg/day for Segment I and III studies in rats or 800 mg/kg/day in Segment II studies in rats (3 studies) and rabbits.

In general these studies showed little significant maternal toxicity or other orlistat associated adverse effects. There was, however, an increased incidence of dilated cerebral vesicles in rat fetuses of a Segment II study which was statistically significant at the 800 mg/kg high dose. This finding was not reproduced in two additional Segment II rat studies or in the Segment II rabbit study. The last or 3rd Segment II rat study utilized both Ro 18-0647/002 (crystalline polymorph A) and Ro 18-0647/008 (fine milled polymorph B), and was performed by an independent contract laboratory. [It is reported that enlarged cerebral ventricles, as opposed to hydrocephalus, can occur as a result of slightly retarded development.] In one study with Ro 18-0647/002 the rate of resorptions was significantly higher in the high dose group but within range of historical controls. The one study showed an increased significant effect on the incidence of 7th cervical ribs in the mid and high dose Ro 18-0647/008 groups and an increased incidence of unossified pubes in the high dose Ro 18-0647/002 group. The incidence of cervical ribs, a common finding in this strain of rats, was within range of that of historical controls. The increased incidence of unossified pubes, although slightly greater than that of historical controls, is a subcategory of retarded pelvic (growth) ossification which did not significantly differ among the dosage groups. In the rat Segment III study the gestation period was increased in one low and one high dose dam which was associated with birth of stillborn pups. Plasma triglycerides were significantly increased at the 100 and 450 mg/kg dose levels in the Segment I study.

The rabbit teratology study showed a single low dose fetus with an umbilical hernia and two mid-dose (300 mg/kg) fetuses with hydrocephalus but there was a lack of a dose-response. Cystic dilatations of brain structures (around the medulla oblongata) found in all groups were considered by the sponsor to be fixation artefacts.

No impairment of physical functional development in the F_1 generation of the Segment III rat study was observed and studies to assess memory and learning in offspring were unremarkable.

Pregnant rats subjected to an autoradiographic study at a dose of 200 mg/kg showed that low, hardly detectable levels of radioactivity were localized in the placenta or fetus.

Toxicokinetic studies in female rats and rabbits at 800 mg/kg, the highest oral dose used in the reproduction studies showed good systemic exposure in rats

(432-813 ng/ml). However, systemic exposure in the rabbit was sporadic and relatively poor. Plasma levels ranging from 9.6-28.9 ng/ml were seen in only 20% of plasma samples collected.

No evidence of mutagenicity or genotoxicity was associated with orlistat in any of the following assays: Ames test; Mammalian cell (V79/HPRT) gene mutation assay; Unscheduled DNA synthesis in primary cultures of rat hepatocytes (UDS assay); Clastogenesis in vitro in human peripheral lymphocytes; Chromosome aberration assay in vivo in mice (mouse micronucleus test).

Orlistat was not antigenic as tested in the guinea pig.

Additional Studies:

Bulk drug substance impurities (Ro 18-6934, Ro 19-1495, and Ro 19-3052) were identified in various lots of orlistat. Acute oral toxicity was studied in rats at limit doses. In addition acute oral and i.v. toxicity of Ro 41-4522/000, a breakdown product, was studied in mice. There was no unscheduled mortality following oral dosing. The NOEL for rats in a 4-week oral study of Ro 19-3052, the next to last intermediate in the chemical synthesis of orlistat, was 200 mg/kg/day. In general findings were of the type seen with orlistat. There was, however, a slightly increased mean relative liver weight for male rats.

Three capsule degradation products, Ro 43-2042, Ro 47-2139 and Ro 61-1227, in the range of 0.1-0.4%, were identified in orlistat capsule lots stored at 25°C and 60% relative humidity. The toxicity of these products was studied in a 13-week oral study in rats, a Segment II rat teratogenicity study and 2 short-term genotoxicity studies. Test substances were pure orlistat and orlistat "spiked" with Ro 61-1227, Ro 43-2042, and Ro 47-2139 at levels of 0.5%, 3.0% and 3.0%, respectively.

In general no significant toxicological differences between orlistat and the spiked compounds were seen. No unexpected or previously unobserved findings were evident. No bacterial mutagenesis (Ames test) or chromosomal aberrations (clastogenesis assay in human peripheral blood lymphocytes) were found.

The toxicity of orlistat prepared by chemical synthesis and by fermentation methods was investigated in 2-week rat studies. Findings were in general similar with the two preparations. Systemic exposure was assessed by measuring unchanged orlistat and its M1 metabolite, Ro 42-3988, in plasma. Toxicokinetically there were no substantive differences between the two orlistat preparations. Relatively low systemic exposure was seen with both preparations. Mean plasma levels never exceeded 500 ng/ml even with the 1000 mg/kg dose. However, systemic exposure for the fermentation product was more than dose proportional (F>M) suggesting increasing GI absorption and/or saturation metabolism at higher dosages.

Labeling: Labeling needs modification.

1) With regard to females in the mouse (Han IBM: NMRI mouse, SPF) carcinogenicity study, two hemangiosarcomas were found only in the livers of two of the high dose group (1500 mg/kg). According to statistical analysis by FDA (HFD-715), the positive linear dose-response trend for liver hemangiosarcoma in female mice indicated that this trend was statistically significant (p=0.0248). A cut-off p-value of 0.025 was used (a criterion set by the Agency), based on the