Appendix 3

CAP 7C0211

ROCHE VITAMINS AND FINE CHEMICALS
HOFFMAN-LA ROCHE INC.

ASTAXANTHIN AS A PIGMENTER IN SALMON FEED

VOLUME 2 OF 7

(D)(1) ASTAXANTHIN: HUMAN FOOD SAFETY SUMMARY

(See Volumes II through VI for complete reports)

In another trial, the acute toxicity of astaxanthin in rats was determined following administration of 10 consecutive daily oral doses ranging from [illegible] to 2000 mg/kg b.w. There was no mortality and no symptoms of toxicity were reported.

Lethal doses in mg/kg p.o.	24 h after [illegible] administration	24 h after 5 days of daily compound administration	24 h after 10 days of daily compound administration	10 days after 10 days of daily compound administration
LD 10	over [illegible]	over 2000	over 2000	over 2000
LD 50	over [illegible]	over 2000	over 2000	over 2000
LD 90	over [illegible]	over 2000	over 2000	over 2000

II. Mutagenicity

a) Ames Test

Astaxanthin at concentrations ranging from 0.03 to 5.0 mg/plate did not induce mutations in Salmonella typhimurium tester strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 with or without activation by a rat liver homogenate fraction.

b) Micronucleus Test

Astaxanthin in the form of the 10% gelatin beadlet was administered orally to mice at 30 hours and again at 6 hours prior to sacrifice. Dosage was 500, 1000, and 2000 mg/kg b.w. of the 10% gelatin beadlets. No compound-related increase in micronuclei was observed. Astaxanthin at the doses tested induces neither chromosome breaks nor mitotic disjunction in vivo.

IV. Reproductive Performance Study in Rats

Astaxanthin was tested in accordance with the guidelines of the American FDA and the English CSM for effects on fertility and general reproductive performance of the rat and on the in utero and postnatal development of the F1-offspring to time of weaning. The study includes the assessment of later development and of the reproductive capability of selected F1-offspring retained beyond weaning.

Doses of 25, 100 or 400 mg/kg/day were administered by oral gavage to 32 male rats/group, beginning 70 days prior to mating and continuing until sacrifice, and to 32 females/group beginning 14 days prior to mating and continuing through gestation until sacrifice or weaning. Control animals (32/sex) received the vehicle (rape-oil, 2 ml/kg) in a comparable regimen.

About half of the mated females in each group were sacrificed on about gestation day 14, while the remaining females were allowed to litter. F1-pups of selected litters were evaluated for developmental indices during lactation. On lactation day 23, selected weanlings were retained for learning and memory testing or the assessment of their reproductive capability.

The results of the study can be summarized as follows:

P-GENERATION

No substance-related mortality in males or females was observed in any of the dosage groups. The body weight gain of both P-males and P-females in all experimental groups matched that of concurrent controls.

The percentage of males which mated their partners, as well as the ratio of mated to pregnant females and the median precoital time were comparable between all groups.

Up to 400 mg/kg, the highest dose tested, the reproductive parameters of females sacrificed between gestation days 14 and 16 were within normal limits.

F1-GENERATION

In all experimental groups, the litter parameters such as the body weight gain of pups, the time of onset of developmental landmarks and the learning and memory ability matched that of the controls.

The neonatal mortality of the F1-generation in the highest dosage group (400 mg/kg) was at the upper limit of the biological range (25.6%). However, there was no statistical significance for this finding and no dose-relationship was evident. Therefore a substance-related impairment of pup viability was considered to be very unlikely.

In all dosage groups, the macroscopic and soft tissue examination of pups found dead during lactation showed isolated findings which were not considered to be substance-related. These included hematoma in the lung, empty stomach, and dilated renal pelvis and ureter. The gross examination of weanlings for malformations, as well as for liver and kidney weights, yielded normal findings. One neonate in the low-dose group (25 mg/kg) exhibited unilateral anophthalmia. This isolated anomaly was not considered to be substance-related.