Male rats were fed diets containing 40% glucose for 23 days, after which the glucose was replaced by DHA. Such replacement caused a reversal of the increase in weight-gain, and food and water consumption was reduced. After 21 days on the DHA diet, 75% of the animals had died. In a further experiment, in which the rats were given a diet containing 20% glucose and 20% DHA, growth was slower than with the 40% glucose diet, and a marked deterioration of the quality of the food was noticeable during the feeding period.

Thus, in the light of this work, ingestion of substantial quantities of DHA in the diet, either during prolonged space missions or in other circumstances, cannot be considered advisable.

2496. Bacterial irony

Nutrition Reviews (1972). Iron and zinc absorption and metabolism in germfree rats. *ibid* **30,** 148.

The idea that intestinal micro-organisms affect the absorption of iron, and possibly zinc, has derived some support from experiments with germ-free animals, in which biochemical signs of iron deficiency in vital organs have often been observed. The review cited above investigates such claims by considering certain balance studies on germ-free rats fed diets adequate in iron and zinc.

In a recent study (Reddy *et al. J. Nutr.* 1972, **102,** 101), the urine and faeces of adult conventional and germ-free rats were collected over a period of 10 days immediately preceding sacrifice. Absorption of iron was about 25% lower in germ-free than in conventional rats, and net retention of iron also decreased, although this was not reflected in any change in the level of urinary excretion. Growth was comparable in both types of rat and there was no significant difference in the degree of absorption or retention of zinc in the two groups. The activities of hepatic xanthine oxidase and renal catalase, both of which contain iron, were markedly lower in the germ-free group, but levels of zinc-containing alkaline phosphatase in the liver, kidney and serum and of catalase in the liver did not differ from those of controls. The zinc levels in the liver, kidneys and femur were the same in both groups, but when calculated per unit of body weight that of the femur was significantly higher in the germ-free group because the femur weight and ash-values were greater. This finding was consistent with the greater calcium and magnesium deposition in the bones of germ-free animals compared with conventional groups and probably reflected the necessary maintenance of a given ratio of mineral components in bone.

It may be that the iron requirement is lower in germ-free than in conventional animals and that this results in a corresponding decrease in absorption. In any event, these results serve to emphasize the important part played by the intestinal flora in normal animal nutrition.

AGRICULTURAL CHEMICALS

2497. Carbaryl toxicity testing criticized

Weil, C. S., Woodside, M. D., Carpenter, C. P. & Smyth, H. F., Jr. (1972). Current status of tests of carbaryl for reproductive and teratogenic effect. *Toxic. appl. Pharmac.* **21,** 390.

Carbaryl (1-naphthyl methylcarbamate) has been shown to reduce the female fertility index when fed at 10,000 ppm in the diet to rats and gerbils, and to have a dose-related adverse effect on litter size (*Cited in F.C.T.* 1972, **10,** 261). It has also been reported to have teratogenic effects in beagles (*ibid* 1969, **7,** 686) and guinea-pigs (*ibid* 1970, **8,** 102).

The authors of the paper cited above report some discrepancies between their own results and other published and unpublished reports on the effect of carbaryl on reproduction and foetal growth. A three-generation study in rats given feed additions calculated to provide a daily intake of up to 10 mg/kg body weight failed to demonstrate any significant dose-related effect of carbaryl on fertility, gestation, viability of pups or lactation. In a later study, pregnant rats were given carbaryl at dietary levels equivalent to doses of up to 500 mg/kg/day without any effect on fertility, gestation or incidence of teratogenic abnormalities. Weight gain was unaffected in rats given 100 mg/kg/day but was reduced in the 500 mg/kg group, in which many of the pups died before being weaned from the carbaryl-fed mothers. Neither teratogenic effects nor adverse effects on fertility or gestation were seen in another rat study in which animals were fed up to 200 mg carbaryl/kg/day, although 100 mg/kg/day given by intubation caused significant mortality, cholinesterase inhibition and a reduction in fertility. Growth was reduced to a comparable extent in both these groups.

These results from US tests are in marked contrast to findings reported from the USSR, where effective doses were found to be much lower and where daily doses were given in a single intubation rather than in the diet. Greater uniformity of results would be expected to result from the universal adoption of guidelines for test protocols. One such set of guidelines (Weil, *Toxic. appl. Pharmac.* 1970, **17** (2), i) involves using a species which metabolizes the test substance in a way similar to man, giving several dose levels (relying on high doses only to define the mechanism of action and on low doses to define the no-effect threshold), applying statistics only to randomly distributed units and administering the substance by a route relevant to the mode of human exposure.

Apart from the three studies reported in this paper, only one study out of the many discussed conformed to all these guidelines and the results showed that over three generations of rats a dietary level of 5000 ppm carbaryl (about 250 mg/kg/day) affected reproduction, whereas a level of 2000 ppm (100 mg/kg/day) did not. The much lower no-effect levels established in the Russian studies were attributed to the method of dosing, which placed the whole daily dose in the stomach at one time unaccompanied by any food and would thus be expected to result in much higher peak levels of carbaryl in the blood than would ever be attained with the more relevant dietary administration.

2498. Chlorodibenzodioxins in the liver

Cunningham, H. M. & Williams, D. T. (1972). Effect of tetrachlorodibenzo-*p*-dioxin on growth rate and the synthesis of lipids and proteins in rats. *Bull. env. contam. & Toxicol. (U.S.)* **7,** 45.

Williams, D. T., Cunningham, H. M. & Blanchfield, B. J. (1972). Distribution and excretion studies of octachlorodibenzo-p-dioxin in the rat. *Bull. env. contam. & Toxicol. (U.S.)* **7,** 57.

The chlorodibenzo-*p*-dioxins have been shown to be causative agents in the chick oedema syndrome (*Cited in F.C.T.* 1967, **5,** 584; *ibid* 1969, **7,** 394). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (I), which has been shown to be a teratogenic contaminant of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (*ibid* 1970, **8,** 596; Emerson *et al. Fd Cosmet. Toxicol.*