2. REVIEW OF LITERATURE

Only extremes of malnutrition have been reported to influence the toxicity of pesticides. However, there are a number of factors which play an important role in evaluating the effect of starvation on the toxicity of pesticides. In case of organochlorine pesticides, the mass of body fat present in the well-fed animal serves as a protective mechanism by storing the pesticides and thus shielding the sensitive nervous tissue from the poison. In the starved animal this protective mechanism is either lost or is not fully effective. The altered liver microsomal enzyme activity of starved animals may also influence their susceptibility to poisoning by pesticides.

2.1 Effect of Starvation

2.1.1 Effect on Body Fat: According to Hayes (1959), various mammals, birds and fishes are relatively resistant to poisoning by DDT if they are fat rather than thin. DDT is usually stored in body fat in sufficient concentration. Rapid mobilization of the fat through starvation may lead to characteristic DDT tremors during starvation (Fitzhugh and Nelson, 1947).
There is an increase in the concentration of pesticide in the small amount of fat left after mobilization and by the same token in all the tissues of the body (Dale et al. 1962). During mobilization of DDT, excretion is increased by a factor of about 1:4, but this increase is insufficient to prevent poisoning in some cases. It was, however, pointed out by the author that starvation is unlikely to precipitate toxic effects of DDT in man. This is because of the fact that even people with heavy occupational exposure to the compound do not store enough of it to produce the toxic effects since the metabolism of man is inherently slower than that of rat and the man cannot starve so fast. In nature, starvation is more often partial than complete. If the original diet contains enough DDT to cause substantial storage, whatever food may be found in a period of scarcity is also likely to be contaminated. The initial effect of the mobilization of fat is to increase the concentration of DDT in the remaining fat and in other tissues. Excretion is increased in response to the increased tissue levels but may not be fast enough to prevent the accumulation of a toxic concentration in the brain. If intake of DDT is stepped, the increased
rate of excretion eventually leads to reduced storage (Dale et al., 1962). These findings have been confirmed in regard to both the initial increase in the concentration of DDT (Dedek & Schmidt, 1972; Stenberg & Diky, 1973) and the later reduction (Brodeur & Lambert, 1973). Similar findings have been reported in birds (Adamczyk, 1971).

The effect of fat mobilization on the toxicity of DDT is the same whether it is caused by withholding food or by disease that causes partial refusal of food (Hayes, 1975). Accumulated fat in the body influences the amount of pesticide that can be stored inactively in the body having a positive role in mitigating acute poisoning. This phenomenon has been noted in connection with mammals (Spicer et al., 1947) and fish (Hoffmann & Surber, 1948). In contrast, laboratory animals are slightly more susceptible to repeated large doses administered as part of a diet containing a moderate proportion of fat than as part of a very low fat diet. Accordingly, both the mouse and the rat showed increased toxic effects of DDT when the percentage of fat in the basal diet was increased from 5 to 15 (Sauberlich & Baumann, 1947).
A reduction in the level of dietary fat to 0.5 per cent decreased the toxicity of DDT in both species. This difference is thought to be associated with absorption from gastrointestinal tract. All lipids tested including a highly saturated fat (hydrogenated coconut oil), moderately saturated fats (butter and lard) and highly unsaturated fats (peanut oil and corn oil), produced essentially similar effects. The effect of fat was not modified by the addition of cholesterol at a dietary level of 0.5 per cent. It has been suggested that the increased toxic effects of DDT given in a high fat diet may be due primarily to greater absorption of the toxicant in the presence of fat.

Starvation has been reported to alter liver microsomal enzyme activity in male mice (Dixon et al. 1960). The decreased enzyme activity associated with starvation appeared to be due to an actual loss in enzyme protein rather than to a deficiency of essential co-factors or to the presence of inhibitors of the drug metabolizing enzymes. Manthei et al. (1964) have reported that restriction of dietary protein also decreased drug metabolizing capacity in mice through lowered microsomal enzyme levels.
The association of lipids with the function of microsomal enzymes is generally recognized as is the fact that DDT induces these enzymes. Therefore, it might have been expected that DDT and essential fatty acids would interact. Tinsley & Lowry (1972) found that the growth of female rats receiving p,p'-DDT at a dietary level of 150 mg/kg was depressed, if they received a diet deficient in essential fatty acids, but was slightly stimulated, if they received the same diet supplemented with these acids. Another variable influenced by the same factors was the ratio of various liver lipids. The changes in fatty acid composition were related to the proliferation of hepatic smooth and endoplasmic reticulum. It was suggested that DDT influenced essential fatty acid metabolism by increasing the demand for them.

In contrast, a variety of diets containing rats equal to the amount found in a typical human diet had little or no influence on the storage of DDT. Similar results were obtained with a wide range of pesticides fed to rats for four generations in a combination of rates 200 times those found in the Market Basket Study of Food in the USA (Adams et al. 1974).
The mobilization of chlorinated hydrocarbon insecticides, due to starvation, differs from compound to compound. For example, Heath and Vandekar (1964) reported that the average excretion of dieldrin in rats (5 per cent per day) was more than doubled following a few days of starvation. It was, therefore, not possible to precipitate dieldrin poisoning in rats by starving them after they had been fed the compound for 7 to 18 months at dietary levels up to 15 ppm (Treon and Cleveland, 1955).

In mirex exposed rats, the rate of excretion of insecticide was unaffected by food deprivation. Although food deprivation causes a relocation of mirex residues, it does not significantly alter the toxicity to an appreciable extent (Villeneuve 1977).

2.1.2 Effect on Enzymes: In squirrel monkeys (and presumably in other species) only two days on an ascorbic acid-deficient diet impaired both the induction of O-demethylase and the stimulation of the glucuronic acid system by DDT (5 mg/monkey/day) (Chadwick et al. 1971). In guineapigs, maintenance of induction of microsomal enzymes required a higher
dietary level of ascorbic acid than prevention of scurvy (Wagstaff, 1971).

2.2 Effects of Dietary Protein

2.2.1 Quantitative Effects: In addition to the action involving storage and excretion referred above, nutritional status may influence toxicity, through metabolism induced by hepatic microsomal enzymes. The effect on protein deficiency on toxicity may involve a crippling of microsomal enzymes of the liver. Murphy and Dubois (1958) reported that male rats maintained on a protein free diet for four weeks had only 24 per cent as much microsomal enzyme activity as normal rats. Also the liver enzymes of rats which did not receive proteins could not be induced by compounds that ordinarily stimulate these enzymes. Administration of parathion in rats maintained on a protein deficient diet, markedly altered the liver function; the treatment resulted in significant reduction in bile-flow and biliary excretion of bilirubin and the bromsulfalein and an increase in the retention of these pigments in the serum (Garay et al. 1975).
There is a great variation in the effect of protein deficiency on susceptibility to acute toxicity by different compounds. This variation may depend on: (i) the process of biotransformation involving detoxication or toxication; (ii) extent of biotransformation related to toxicity; (iii) anorexia of interference with nutrition caused by some compounds; and (iv) by other mechanisms or a combination of above.

For instance, the protein deficiency may influence the biotransformation of certain compounds; the toxicity of aflatoxin, which is detoxified by the liver, is increased by protein deficiency; while the toxicity of carbon tetrachloride is considerably decreased by protein deficiency (McLean and McLean, 1969).

Smith & Stohlman (1945) found only slightly greater mortality and liver pathology in rats fed DDT at 500 mg/kg in a diet containing protein at 80 gm/kg than in one containing 280 gm/kg. The finding that low dietary protein predisposes to DDT poisoning has been confirmed (Sauberlich & Baumann, 1947) Boyd & DeCastro, 1968, 1970; Boyd & Krijnen, 1969). The acute toxicity (LD$_{50}$) of DDT and its clinico-pathological
effects were only slightly changed in rats maintained for four weeks on a synthetic diet deficient in proteins (8 per cent casein) or normal proteins (27 per cent casein) (Boyd and DeCastro, 1968).

Even on a diet without proteins, the toxicity of DDT was increased only four fold (Boyd and Krijnen, 1969b), while that of malathion was increased two fold (NIOH, 1982). By contrast, the acute toxicity of captan was increased 2,100 fold in rats maintained on a diet without proteins as compared to those fed normal protein diet ((Krijnen and Boyd, 1970). The LD$_{50}$ of endosulfan as determined in rats fed on diets containing 0%, 3.5%, 9%, 26% or 81% protein as casein was 5.1, 24.1, 57.0, 102.0 and 98.0 mg/kg respectively (Boyd et al., 1970 a&b). The results for DDT, captan and certain other pesticides are summarized in Table 3. It may be noted that even a considerable increase of proteins in diet only moderately influenced the toxicity of pesticides. And vice versa, a reduction of proteins in diet to one third of normal, only marginally increased the toxicity of certain compounds. However, with gross protein deficiency, susceptibility to toxic effects of certain pesticides is increased
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The concentration of radioactivity (counts per minute) of serum particulates in animals at 25 weeks after exposure to the organophosphorus pesticides in the diet was determined to be 2.3 ± 2.1 ppm. The concentration of radioactivity (counts per minute) of serum particulates in animals at 25 weeks after exposure to the organophosphorus pesticides in the diet was determined to be 2.3 ± 2.1 ppm. The concentration of radioactivity (counts per minute) of serum particulates in animals at 25 weeks after exposure to the organophosphorus pesticides in the diet was determined to be 2.3 ± 2.1 ppm.
significantly. Rats maintained on a protein free diet for 28 days from weaning weighed about 30 per cent less than those maintained on the usual diet. Two thirds of these rats died in the first few days after withdrawal of usual diet. The susceptibility of these animals to compounds or toxicity may be related to anorexia resulting from their administration.

Other compounds which have been studied in relation to protein deficiency, include banol, parathion and chlordane (Casterline and Williams, 1969).

The effect of dietary proteins on the sub-acute oral toxicity of dieldrin has been studied in rats (Lee et al. 1964). A low-protein diet (10 per cent) enhanced the toxic effects of dieldrin as manifested by increased mortality, increase in liver lipids, decrease in total hepatic content of vitamin A and marked histopathological changes involving cellular edema and fatty infiltration. However, the weight of liver in dieldrin-fed rats was not changed by a low protein but was increased by a high protein (25 per cent casein) diet.
A dietary deficiency of riboflavin or nicotinic acid may enhance dieldrin toxicity in rats (Tinsley, 1966). Also, dieldrin appears to interact in the metabolism of unsaturated fatty acids and accentuate an essential fatty acid stress.

Dietary DDT at levels of 10-100 ppm decreased the utilization of vitamin A and carotene in the rat as measured by hepatic storage of vitamin A (Philips, 1963).

In a review of diet and toxicity, McLean and McLean (1969) described the effects of protein deficiency on the toxicity of compounds which are detoxicated, particularly those by biotransformation and those where the biotransformation occurs at the site of toxic injury. They pointed out that reversal of one aspect of deficiency (such as the induction of microsomal enzymes by a foreign compound or by a compound of natural diets in animals with borderline protein deficiency) may reverse the entire effect of diet on toxicity although there is evidence that malnourished people are unduly susceptible to infection. They did not find a clear evidence that the ability of the cell to withstand stress and trauma in general is altered by malnutrition.
2.2.2 **Qualitative Effects:** The oral toxicity of heptachlor was reported to be 1.6 to 2.1 times greater in rats fed casein than in those fed gluten, irrespective of the concentration of protein (10 to 18 per cent) in the diet. The gain in weight was greater and the difference in toxicity was less or even reversed when the animals were fed casein diet ad libitum (Webb and Miranda, 1973). Gluten is an incomplete protein that reduces food intake and permits only a small increase in body weight of rats that consume it ad libitum as their only source of protein. It seems likely that the lower toxicity of heptachlor in rats fed gluten depends on a small conversion of the compound to heptachlor epoxide as a result of limited activity of the microsomal enzymes of the liver. On the other hand, the greater protection afforded by normal intake of high quality protein may result from the presence of normal fat deposits and the sequestering of both heptachlor and its epoxide in the fat.

2.3 **Miscellaneous Nutritional Effects**

Deficiency of any essential trace element may be injurious by itself. However, a minor deficiency
may predispose to injury by a toxicant. Furthermore, there may be an interaction in the metabolism of trace elements whether essential or not. For instance Brinkman and Miller (1961) found that rats fed molybdenum gained less weight and had lower hemoglobin levels if they were kept in galvanized instead of stainless steel cages. Similar effects were observed by increasing the zinc content of the diet of rats fed molybdenum and kept in stainless steel cages.

2.4 Enzymatic Studies

A number of enzymes of intermediate metabolism are either stimulated or moderately inhibited by toxic doses of DDT (Agarwal, et al. 1978a; Arvindakshan et al. 1977). Administration of 800 ppm of beta- and gamma-isomer of HCH to weanling rats significantly altered the activities of various enzymes of kidney and liver (Srinivasan et al. 1977). The effects of endosulfan on various enzymes involved in pentobarbital metabolism revealed that pretreatment significantly increased the hepatic microsomal enzymes responsible for pentobarbital metabolism (Agarwal et al. 1978b). The distribution pattern of endosulfan in plasma and brain tissue was studied when rats were fed daily doses of endosulfan (5 or 10 mg/kg) per 15 days.
The concentration of α-isomers was in the order of cerebrum > remaining parts of brain > cerebellum. The β-isomer was not detected in the remaining part of the brain. On a per unit lipid basis, the concentration of endosulfan in cerebrum was one and a half times that of the whole brain tissue and might be due to the higher lipid content in the cerebrum than in the rest of the brain. Endosulfan sulphate was the only metabolite detected in the rat brain (Gupta, 1978). Of the organophosphate pesticides, malathion administered intraperitoneally at 50 mg/100 g body weight induced significant increase of blood glucose and plasma sodium during the first six hours of treatment while the glycogen content of the liver, kidney, heart and spleen showed a marked increase during the next 6-24 hours (Gupta, 1974).

2.5 Histopathological Studies

The prominent symptoms of pesticide poisoning are hyperactivity, tremors and convulsions of clonic nature followed by death. Other signs of poisoning are decreased respiration, dyspnoea and salivation. Espir et al. (1970) and Peck (1970) have observed
Impotence among farm workers. Lang (1971) also reported a variety of physiological disorders and illness among workers associated with the application of pesticides. Several histopathological studies on sensitive body tissues following exposure of commonly used pesticides have been done (Kelly-Garvert and Legator, 1973; Collins et al. 1971; Mrak, 1969; Legator et al. 1969; and Palmer et al. 1972).

Histopathological studies done in rats and mice using endosulfan revealed damage to the liver, kidney and testes (Gupta, 1976). Consistent histopathological changes were apparent only at a dose level of 5 mg/kg which produced renal tubular damage. Kimbrough et al. (1972) have reported hepatic changes like Kupffer cell hyperplasia and bile duct proliferation after single and repeated exposure to several pesticides. Other workers (Ortega et al. 1957) have also observed liver cell changes following exposure to various pesticides.