

THE PROBLEM OF RESIDUES OF CHLORINATED
HYDROCARBONS AND ORGANIC PHOSPHORUS
COMPOUNDS IN MEAT AND MILK.

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List of abbreviations

ADI	:	admissible daily intake
BHC	:	benzene hexa chloride
DDA	:	1,1 dichloro - 2,2 bis (p-chlorophenyl) acetic acid
DDD	:	1,1 dichloro - 2,2 bis (p-chlorophenyl) ethane
DDE	:	1,1 dichloro - 2,2 bis (p-chlorophenyl) ethylene
DDT	:	1,1,1 trichloro - 2,2 bis (p-chlorophenyl) ethane
HCB	:	hexachlorobenzene
HCH	:	hexachlorocyclohexane
L _D 50	:	average lethal dose
ppb	:	parts per billion
ppm	:	parts per million
PRS	:	Pesticide Residues Series
TOCP	:	tri-o-cresyl phosphate
TRS	:	Technical Report Series
WHO	:	World Health Organization

ABSTRACT

The metabolic and toxicological characteristics of chlorinated hydrocarbons and organic phosphorus compounds are reviewed.

Chlororganics accumulate in fat depots and lipid fractions of body tissues and milk; accumulation patterns present remarkable difference among the various compounds.

Depletion of organochlorines from tissues or milk is slow and several months can be required for the residues to disappear. These compounds show a wide range of chronic effects on several metabolic systems in animals.

Organophosphates, even the lipophilic ones, do not show accumulation and/or persistence of residues to an extent comparable to that of chlororganics. However they are worthy of attention because of their high toxicity and their very widespread use.

With regard to presence of residues in food, some methods of sampling and prevention are reviewed. It is pointed out that the main aim of monitoring should be to furnish data on quantitative and qualitative features on environmental contamination from pesticides and on their misuse.

Public health risk from pesticide residue in foods should not be overemphasized, provided that a good control on pesticide use is achieved.

INTRODUCTION

During the last few decades the greatly intensified use of pesticide chemicals to combat disease and to increase food production has brought about considerable benefits both economic and sanitary. It has, however, created some potential public health problems.

Environmental pollution, arising mainly from the more persistent compounds, has been recognized as a possible cause of disruption of food webs and ecological balances and as an actual cause of mass mortality among wildlife (Edwards, 1975). Besides this, farm animals come often in contact with pesticides, through environmental contamination and through treatments, and residues are very frequently found in foods of animal origin.

Chlorinated hydrocarbons are the most important from the point of view of food pollution, because of their persistence, both outside and inside living organisms, leading to the possible presence of high level, long lasting residues, which might have a wide range of chronic effects, as demonstrated in several animal species.

Many of the organophosphorus pesticides are not persistent and most are unlikely to accumulate in organism or along food chains: however, they are worthy of attention and of investigation because they are highly toxic and possibilities of misuse, leading to the presence of residues in human food, always exist. Moreover, they are now, by far, the most widely used class of pesticide and, as they gradually substitute chlorinated hydrocarbons, there is some evidence that organophosphate traces in foods are increasing (Edwards, 1975).

According to Grossklaus (1978), food, mainly that of animal origin, is the most important way of introduction of pesticides into the human body, a very significant intake coming particularly from meat and milk. These food items can be taken as an important and representative part of the whole problem and this dissertation will deal in particular with them.

LITERATURE REVIEW

1) Chlorinated hydrocarbon compounds

a) General characteristics

All the chlororganic pesticides are prepared by chlorination, to an extent generally corresponding to about 33-67%, of various hydrocarbon compounds (Clarke, Harvey and Humphreys, 1981). The chemical features of each compound have been described in detail by Ware (1978), who, according to chemical structure, divided them into several groups: (a) the di-phenyl aliphatics, which include DDT (1,1,1 trichloro - 2,2 bis -p-chlorophenyl ethane), its two isomers (p-p' and o-p') and its derivatives DDD (1,1 dichloro - 2,2 bis -p-chlorophenyl ethane), methoxychlor and others. (b) The benzene derivatives, including BHC (benzene hexachloride), also called HCH (hexachlorocyclohexane), and its 5 isomers (α , β , γ , δ , ϵ); lindane, which is composed 99% γ BHC, the only isomer with real insecticidal power; and the closely related HCB (hexachlorobenzene), a fungicide.

(c) The cyclodienes, which include aldrin, chlordane, dieldrin, endrin, heptachlor, kepone, mirex and others of minor importance.

(d) The polychloroterpenes, which have been discovered only after the Second World War and include toxaphene (also called camphechlor) and strobane. Features shared by most of these compounds, and which have a bearing on residue problems, are their high resistance to most physical and chemical factors and their very low hydrosolubility (DDT is perhaps the most water-insoluble chemical ever synthesized: about 6 ppb).

b) Persistence and metabolism

Environmental persistence is a striking feature of organic chlorine pesticides: Viviani (1973) reported that, for instance, BHC persists in the environment for 11 years, DDT for 10 years, heptachlor for 9 years, dieldrin for 6 years, toxaphene for 5 years. Their strong lipophilic properties allow them to be removed from water by the smallest amount of organic debris and to be assembled by living organisms, leading to progressive accumulation of the compounds and their metabolites in food chains.

Problems arising from the effects of the environmental burden of pesticides on wildlife and ecological balances, which are beyond the limits of this dissertation, have been dealt with in detail by Edwards (1975) and Spencer (1971).

In food producing animals, contamination arises mainly from ingestion, but also from cutaneous and respiratory intake. Absorption from the gut is poor (Clarke et al., 1981) and faeces may be an important way for recycling the compounds. Kan and Jonker-den Rooyen (1978b), stated that hens fed with different organic chlorines excreted up to 80% of the ingested dose through eggs and faeces. Avrahami and Steele (1972) suggested that significant contamination observed in controls which grazed with HCB-treated sheep arose, most probably, through the excreta. Arthur, Cain and Barrentine (1975) reported that rabbits exposed for 3 months to the atmosphere of an area where usage of pesticides was high had much higher residues in their adipose tissue than those exposed to the atmosphere of a low usage area: the former animals had 20 times greater DDT, 7 times DDD, 5 times DDE, 10 times total DDT and 2 times dieldrin and

heptachlor than the latter. Even a drift of dust derived from treated flocks could give detectable levels of residues, albeit very small, in untreated animals (Putnam, Brewer and Cottier, 1974).

After absorption into the body, most compounds undergo hydroxilation in the liver and metabolites are formed, which are also persistently stored in the tissues. Metabolism of DDT varies in different species, perhaps because of the different digestive floras. In ruminants, 79% of DDT is converted in the rumen into DDD within 48 hours, DDA (1,1 dichloro - 2,2 bis p-chlorophenyl acetic acid) is then formed and, eventually, chlorophenyl acetic acid, which is excreted in urine (Fries, 1977; Richou-Bac and Cumont, 1971). In man, 75-80% of DDT absorbed from gut is excreted as DDA (Clarke et al., 1981). In birds the main metabolite is DDE (1,1 dichloro - 2,2 bis p-chlorophenyl ethylene) (Richou-Bac and Cumont, 1971); it is noteworthy that also in ruminants DDE, though a minor metabolic pathway, gives the greatest residue levels, DDD being moderately present only in fat and liver (Whiting, Brown and Stull, 1973). Conversion into DDD can be regarded as a metabolic expedient and excretory pathway, because it is a product less subject to storage and more readily excreted through faeces (McBlain, Lewin and Wolfe, 1974).

Most aldrin is converted, in the body, into dieldrin; most heptachlor into heptachlor epoxide, which is more toxic: so, with regard to heptachlor, a diet low in protein, which depresses hepatic metabolic activity, has a protective effect (Naber, 1977).

Dieldrin, heptachlor epoxide and, less markedly, DDE as well, are stored in the body more efficiently than the original compounds (Naber, 1977). While accumulation generally occurs in adipose tissue and lipid fractions of parenchymatous organs and of muscle also, remarkable differences exist among the various compounds. Blunt and Saunders (1978) found that DDT residue levels showed between different fat depots in cattle; on the contrary, DDE, and HCB as well, showed higher residue levels in perirenal fat, and lower levels in pericardial fat, than in omental or mesenteric depots; fat extracted from retail cuts of meat, heart and kidney showed average levels, but that from liver was 5-fold more contaminated. Dieldrin also showed variations, with highest levels in pericardial fat. These differences may be explained by differences in blood supply and/or different rates of fat deposition and mobilisation, while the liver results may be explained by different lipid composition and metabolic activities (Fries and Marrow, 1977). In lactating cows, DDT and its metabolites have been found in greater amounts in udder than in liver or kidney, while very small traces were present in muscle, ovary, brain, lymph nodes (Whiting *et al.*, 1973) and in placenta (Whiting, Brown and Stull, 1972). DDD and lindane accumulate in adrenals also, at the same concentration as in fat (Clarke *et al.* 1981).

HCB, in swine, showed significant accumulation in fat and bone marrow (Hansen, Simon, Dorn and Teske, 1979) but occurs also, at much lower concentrations, in liver, then in brain and kidney (Tonkelaar, Verschuuren, Bankovska, Vries, Kroes and Esch, 1978).

In broilers residues were found, in decreasing order, in fat, heart, gizzard, leg muscle, kidney, liver, breast muscle (Reed, Booth, Bush, Goetsch and Kiker, 1977), while, according to Avrahami and Steele (1972), the relative amounts in pullets were fat 175, liver 4 and muscle 1.

According to Richou-Bac and Cumont (1977), heptachlor epoxide, in cattle, accumulated abundantly in fat, but produced no residues in muscle and only traces in liver: it appeared that, in normal conditions, it passes with difficulty from adipose tissue to other compartments (e.g. milk). However, in broilers, fat extracted from liver and muscle contained respectively 3.5 and 1.7 times more heptachlor epoxide than adipose tissue did: heptachlor itself was highest in lipid fraction of muscle (Wagstaff, McDowell and Paulin, 1980).

Chlordane is present at significant levels in fat only (Onley, Giuffrida, Watts, Ives and Storherr, 1975). Methoxychlor does not accumulate even in fat and, owing also to the very low toxicity, it is almost devoid of importance in food hygiene (Richou-Bac and Cumont, 1971).

Differences also exist among species: for instance, in India, goats and buffaloes showed residues in bone marrow markedly higher than in fat (Kaphalia and Seth, 1981).

It is surprising to note that, although the nervous system is a classical target for organochlorine toxicity, residues in brain are quite low (Kaphalia and Seth, 1981; Latimer and Siegel, 1977; Whiting et al., 1973).

Accumulation in tissues is dose-related and shows, for each compound, a quite constant ratio between the intake and the stored residue. A remarkable feature is that this relationship holds only until a certain concentration, when an equilibrium is usually reached and no further residues accumulate, no matter how much is added to the diet. This balance between intake and elimination, or metabolism, remains constant as long as intake remains constant; if the intake changes, the amount stored in the body will change also, but not the ratio intake: residues (Edwards, 1975; Naber, 1977; Waldron and Naber, 1974). This "plateau" level has been seen also in human volunteers administered with dieldrin, whose residues in body fat seemed characteristic for each particular daily intake and for each particular individual (Edwards, 1975). Poultry reach plateau levels approximately after 6-12 weeks of exposure (Waldron and Naber, 1974). After cessation of the exposure, depletion from tissues is slow. Half-lives for persistent compounds are generally in a range of 40 to 70 days (Fries, 1977; Kan and Jonker-den Rooyen, 1978b). In poultry, according to Siegel and Latimer (1981), accumulation in males is more long-lasting and reaches higher levels than in females, and more so in entire than in castrated males. This has been explained by a dilution effect, due to the more abundant body fat of females and castrates, leading to a lower concentration of chlororganics. Kan (1978) suggests that the dilution effect influences depletion rate in growing broilers, while in hens depletion is simply correlated with the cumulative potential of the compounds.

In sheep, pregnancy did not affect DDT depletion, but dieldrin concentration was twice that in non-pregnant animals: after parturition, both compounds decreased steadily in body fat, faster in lactating than in non-lactating ewes (Varela Alvarez, 1973).

Several studies have been performed in different species concerning the ratio between concentration in fat and concentration in feed for the various compounds. In poultry, the most studies species, high ratio values are shown by total DDT (9-13), β BHC (14-15), HCB (11-18), dieldrin (11-15), endrin and metabolites (11), while for heptachlor epoxide very variable values are given, ranging from 4 to 20; comparatively little storage is shown by α and γ BHC (1-3) and chlordane (1-5) (Hansen, Dorn and Beamer, 1979a, Kand and Jonker-den Rooyen, 1978a; Kan and Jonker-den Rooyen, 1978b; Kan and Jonker-den Rooyen, Tuinstra, Ros and Trag, 1978; Onley et al., 1975; Reed et al., 1977; Richou-Bac, Restitut and Pantaleon, 1975; Vos, Bouwman and Engel, 1972).

In cows the ranges for the ratio values of heptachlor epoxide, dieldrin, γ BHC are respectively: 6.8-14; 1.12-4.5; 0.8-1.5 (Richou-Bac et al., 1975).

Sheep tend to store strongly HCB (ratio values of 7-18), while ranges for ratio values of γ BHC and dieldrin are respectively 0.41-2 and 1.05-4.8 (Richou-Bac et al., 1975).

In pigs, HCB ratio value ranges from 5 to 7 (Hansen et al., 1977), while ratio value for heptachlor epoxide is 3, that of dieldrin is extremely variable (0.2-7.33) and chlordane undergoes a minimal storage (Richou-Bac et al., 1975). Of course, for most

compounds, ratios smaller or greater than those above mentioned have been occasionally seen.

With regard to storage characteristics of the single chlororganics, Richou-Bac and Cumont (1971) reported that total DDT residues could persist for several months after feeding or cutaneous treatment. According to Foster, Morley, Purkayashta, Greenhalgh and Hunt (1972), DDE residues not only are more persistent than those of DDT, but they tend to increase even after the end of the exposure, owing to DDT metabolisation. DDE traces were more persistent in liver and muscle than in fat (Waldron and Naber, 1974). A marked difference was seen in poultry between o'-p and p'-r DDT with ratio values, respectively, < 1 and 9 in the same trial (Onley et al., 1975).

β BHC, as stated before, is a very cumulative and persistent compound, compared with the other isomers: feeding a diet free from BHC to contaminated chickens for 11 weeks caused a great decrease in α and γ , but not β , BHC (Kan and Jonker-den Rooyen, 1978a), while lindane, administered to hens at 0.1-0.5 ppm/feed, appeared in fat after 6 weeks of feeding and disappeared just 3 weeks after withdrawal (Foster et al., 1972).

HCB seemed to be concentrated more effectively at lower than at higher feeding levels; it reached plateau level in poultry quite quickly, within 38 days (Hansen et al., 1979a), while, on the other hand, its half-life in sheep and steers ranged from 70 to 128 days (Fries and Marrow, 1977). Decrease of heptachlor epoxide was more rapid in liver, and slower in muscle, than in fat, according to Wagstaff et al. (1980): depletion from tissue appeared

to be linear but very slow, allowing long persistence of traces (Waldron and Naber, 1974).

In poultry, aldrin, measured as dieldrin, disappeared slowly from tissues, while dieldrin itself seemed to decrease rapidly after withdrawal from the feed (Waldron and Naber, 1974).

Mirex did not lead to major residues problems in ruminants, even if consumed daily (Bond, Woodham, Ahrens and Medley, 1975), while in broilers it was sparingly excreted and its decrease after withdrawal was mostly due to dilution effect (Ahrens, Dyer and Lloyd, 1980).

Polychloroterpenes are not very persistent. Toxaphene is readily metabolised, poorly stored and rapidly eliminated when input is stopped, although traces may persist in body fat for over 3 months (Clarke et al., 1981; W.H.O. Pesticide Residues Series, 1974).

Transfer of chlorinated hydrocarbons from body fat to milk fat is, of course, a major concern for the food hygienist. According to Clarke et al. (1981), milk is an important excretory route for DDT, while for BHC, dieldrin and chlordane it is a minor one and is insignificant for toxaphene. However, cows administered with BHC, dieldrin and heptachlor showed a sharp increase of residue levels of the first two compounds in milk, while heptachlor was still barely detectable (Vreman, Tuinstra, Hoek, Bakker, Roos, Visser and Westerhuis, 1976). Richou-Bac et al. (1975) reported that the values of the ratios of residue in milk fat to concentration in the feed were high, in cow milk, for dieldrin and heptachlor epoxide (9 and 12.2 respectively), while for } BHC

was only 4.3. In sheep milk the ratio values were respectively 6.25, 9.8 and 8.3, while for HCB it was 4.1; in goat milk β BHC transfer was the most important, with a ratio value of 7.1, compared with 5.1 for HCB and 2.5 - 3 for dieldrin and heptachlor. The values of α BHC ratios in the three species ranged from 2.07 to 5.5, being highest in cows, and are even lower for γ isomer (0.6 - 1.07).

According to Fries (1977), the ratio between milk fat and body fat residues is quite constant for each compound, and transfer from body fat to milk is greatest for dieldrin and HCB, while is markedly low for DDE.

As in the body, a maximum butterfat residue level is reached, in about 15-20 days in sheep, 30 days in goats and 30-40 days in cows (Richou-Bac et al., 1975). Milk fat production is the limiting factor in chlororganic excretion, variation in milk fat production, within the same individual, producing variations in the amount of residues without changes in concentration (Vreman et al., 1976). However, a dilution effect due to increased milk yield has been described, for DDT, by Whiting et al. (1973). They also reported that milk fat residues: concentrations in feed ratios for total DDT (mostly DDE; DDD was very scantily present and almost disappeared at the beginning of the second lactation) ranged from 2.2 (for a 1000 ppb/feed intake) to 3.5 (for a 250 ppb intake): residues originating from the lower intake also showed a longer half-life (respectively 7.3 and 4.4 months).

Mirex did not show a massive excretion in cow milk (Bond et al., 1975). Congenital transfer of residues also occur,

leading to wider diffusion and longer biological life of the contaminants. Calves born from cows fed with DDT (Whiting et al., 1972a) or Mirex (Bond et al., 1975) presented concentrations in body fat and tissues similar to the maternal ones.

Placental transfer of DDT and dieldrin occurred in ewes, according to Varela Alvarez (1973) and resulted in contamination of all the lambs; sucklings stored more residues than non-sucklings, however both showed a gradual decrease in residue concentrations, owing not only to the dilution effect of growth but also to actual metabolism. On the other hand, dieldrin in pigs showed a poor placental transfer, residues in fat, liver and brain of piglets being far lower than those in the sows (Vzoukwu and Sleight, 1972).

HCB was passed by the dams to the offspring either placentally, via the egg or through the milk: residues are high and, at least in piglets, higher than in the dams: newborn may retain their burden for many years (Avrahami and Steele, 1972; Hansen et al., 1979b; WHO PRS, 1974).

Chlororganic residues, carried over by the chickens from the hens, did not seem to influence further accumulation ratios, when the animals were again exposed to organochlorine intake.

c) Toxicity

The targets of organic chlorine acute toxicity are the brain and peripheral nerves. Acute signs may arise after mobilisation of body fat stores, where the toxicant is accumulated.

The oral LD⁵⁰ (average lethal dose) in the rat is very low for endrin (3 mg/Kg body weight) and is also quite low for aldrin, dieldrin, heptachlor and toxaphene (about 40 mg/Kg body weight), while for lindane and DDT it is respectively 76 and 87 mg/Kg body weight. The other compounds show lower toxicity: BHC 125 mg/Kg, Mirex 235 mg/Kg, chlordane 283 mg/Kg; the highest LD₅₀ are shown by DDD (3.400 mg/Kg), methoxychlor (5000 mg/Kg) and HCB (10000 mg/Kg) (Ware, 1978). It is extremely unlikely that residues in human food would occur at levels great enough to produce overt, acute signs: generally, the food hygienist is concerned with chronic effects.

Long-term, low level intake has a recognized effect on liver. The hepatic cells show proliferation of smooth endoplasmic reticulum and a marked increase in activity of microsomal enzymes; however, the limit between toxic effect and adaptive response is still uncertain (WHO Technical Report Series, 1971). Copeland and Cranmer (1974) reported that increased enzymatic activity led to an enhanced metabolism of steroid hormones and drugs. Tseng and Mentzer (1974) observed that dieldrin modified toxicity of organophosphorus pesticides through enhanced metabolism, according to the greater or lesser toxicity of the metabolites compared to the original compounds. Tonkelaar et al (1978) reported

that 0.5 ppm of HCB fed to pigs for 90 days caused an increased enzymatic response and at 5 ppm hepatomegaly was observed. Walker, Stevenson, Robinson, Thrope and Roberts (1969), during a long-term toxicity study, observed that levels of dieldrin as low as 1ppm/feed caused increased liver weight in female rats and dogs, and histological lesions in female rats at 10 ppm, whilst males of both species appeared markedly more resistant. In poultry, liver stores of glycogen and vitamin A were decreased by 10-150 ppm/feed of DDT or dieldrin (Kan et al., 1978).

HCB was associated with porphyrin metabolism disruption at levels as low as 0.5 ppm/feed for 90 days and, often, with ultra-violet fluorescence in livers (Hansen, Wilson, Byerly, Sundlof and Dorn, 1977; Tonkelaar et al., 1978). Photosensitization arose in humans exposed to high doses (Richou-Bac et al., 1975). Lindane, also, produced deposition of ultra-violet fluorescent material in bones of naturally exposed sheep (Nichols, Elsbury and Rousseau, 1981). According to WHO (PRS, 1974) it can also cause blood dyscrasias on an idiosyncratic basis in man.

Reduction in egg shell thickness, the most publicized effect of chlororganics, is generally a problem of wild, mainly predatory, birds (Edwards, 1975). In most experimental studies in domestic animals, such as the study on long-term toxicity of dieldrin performed by Walker et al. (1969), no effect on growth rate or reproduction could be seen. However, in poultry, organic chlorine levels above 50 ppm/feed have a definite depressive action on hatchability and survival of chicks (Naber, 1977).

Besides enhanced hydroxylation of steroids, several adverse effects on endocrine systems are recognized. Concentrations > 50 ppm/feed of aldrin or ≥ 100 ppm/feed of DDT have shown a depressive action on thyroid activity in birds (Naber, 1977); DDD, at 20-50 mg/Kg body weight, caused severe lesions in adrenals in dogs; in man, though it accumulates in the glands, it did not seem to cause any lesion (Clarke et al., 1981). Copeland and Cranmer (1974) observed adrenal functional hypertrophy, caused by the increased steroid metabolism, in dogs: it was accompanied by histological lesions.

Toxaphene lacks reproductive effects, however it shows, at least experimentally in birds, a disruptive action on bone growth and egg shell thickness after continuous feeding of ≥ 5 ppm (Page, Fletcher, Vezey, Bush and Booth, 1978; WHO PRS 1974). Chronic nervous troubles (irritability, occasional convulsions) were seen in rats fed 10 ppm/feed of dieldrin for ≥ 3 months (Walker et al., 1969).

Much controversy has arisen about DDT oncogenicity: 36 ppm/feed for 15 weeks led to the development of unregressable hepatomas in mice; 2 ppm/feed (i.e. 100-fold the average human intake) led to an increase in tumour incidence only in males of one of the two mouse strains tested. Other species tested showed no increased tumour incidence. These findings were thought to have been influenced by too many factors, mainly the proneness of mice to liver neoplasms, to be of real significance for human health (WHO TRS 1971, 1974, 1975). The above considerations can be valid also for hepatomas produced in mice by high doses of lindane,

but not in any other species (WHO TRS 1971). Walker et al (1969) did not observe any rise in neoplasm incidence in rats and dogs administered with dieldrin for 2 years.

Attempts to find correlation between concentrations of DDT and its metabolites in human body and some pathological conditions (cancer, hypertension, liver disease) have generally failed (Edwards, 1975).

According to Grossklaus(1978),it can be concluded that, although risks from human chronic exposure to chlororganics should not be overemphasized, they are worthy of attention because of their potential, insidious actions on several metabolic systems.

d) Residues in meat and milk

According to different agricultural practices and conditions, and also to different regulations about pesticide use, contamination of animal products presents variable features.

With regard to intake through feed, which is believed to be the more important, Crisetig, Mora and Viviani (1975) reported that, in Italy, the compounds found most commonly in feedstuffs were lindane and BHC (probably connected with treatment of harvested crops and storage places), dieldrin and heptachlor epoxide (both resulting mainly from treatment of soils intended for maize and sugar beet) and DDT. Hay and green forage are generally unimportant as sources of intake, unless coming from fruit growing areas or grown in rotation after maize or sugar beet. According to Begliomini and Fravolini (1973) fish meals are contaminated by fewer pesticides, but to a greater level than corn, soy or barley meals; the highest mean value was shown by lindane in fish meals (0.1149 ppm). On the other hand, Moore, Bruce, Kuhlman and Randell (1973), in an investigation carried out on milk contamination by dieldrin in Illinois, showed that the chance of residues exceeding 0.3 ppm was greatest in farms having histories of soil treatment within the last 6-7 years with aldrin. They also reported that hay and oat straw also contained significant amounts, while corn silage, commercial concentrates and well water were of little importance.

A survey performed in U.S.A. by Waldron and Naber (1974) on poultry feedstuffs showed that concentrations of individual chlororganics ranged from 10 to 50 ppb, although, occasionally,

higher values could be seen. DDT and its metabolites were the most frequent pollutants. Fish meal was by far the most contaminated diet item, while corn and soy meals (two major components of broiler diets) showed much smaller residues. Contamination was very closely correlated with areas of crop production and high pesticide usage.

In Italy, cheese made from milk of cows grazed in the lowlands had 0.4203 ppm of total organochlorines on a whole matter basis, compared with 0.0863 ppm of mountain cheese. Differences were found also between milk from an orchard area (0.42 ppm/whole matter) and milk from an area with more traditional cultivations (0.154 ppm), although the two areas were geographically close. Such variations could not be found in beef or chicken fat (Campanini, Maggi and Artioli, 1980).

Rousseau, Pourtailler and Taliencio (1972) have compared, in poultry, residues arising from addition of variable amounts of pesticides to feed and those from disinfection of houses and found the latter to be greater. They suggested that this was due to cutaneous absorption immediately after treatment, followed by ingestion during the following weeks or months, as the birds are accustomed to pick from the floor and the walls. Siegel, Latimer, Drury and Loy (1976) suggested that birds on concrete floors can become more contaminated than those on wire, as they have more possibilities to recycle pesticides in excreta and spilled feed.

HCB residues are generally due to environmental pollution by industrial wastes or by its use in seed-dressing (WHO TRS, 1975).

Many trials have been performed with the object of determining residue levels of various compounds following different administration routes and here it is sufficient to report some data.

After 16 weeks of feeding \leq 0.1 ppm each of mirex, DDT, dieldrin and heptachlor, in combination, to laying hens, the concentrations in body fat of chicks hatched from these hens were respectively 0.47 ppm, 0.049 ppm, 0.024 ppm and just 0.001 ppm, at 1 day of age (Driver, Brewer and Cottier, 1976).

A mixture of 88% DDT and 12% DDE, administered for one year to dairy cows at 250-1000 ppb/feed, led to storage of 700-2000 ppb in renal and udder fat and 40-130 ppb in udder tissue, while liver and kidney showed 10-40 ppb only (Whiting et al., 1973). In calves born from these dams the greatest traces, ranging from 900 to 1400 ppb, were in renal fat, followed by the liver with 30-80 ppb (Whiting et al., 1972a).

In poultry houses where thermal vaporisers for lindane operated continuously or intermittently for 14 months, residues in birds were closely related to the levels of the pesticide in the vaporisers, reaching respectively up to 46 ppm and up to 6-7 ppm on fat basis in tissues (Fishwick, Hill, Rutter and Warre, 1980).

Mirex, administered to hens for 39 weeks at 0.01 or 1 ppm/feed, gave residues in body fat of, respectively, 0.3 and 24.79 ppm: after administration of the same doses for 27 weeks followed by 13 weeks of withdrawal, residue levels showed a moderate decrease

and were 0.28 and 15.5 ppm in the body fat respectively. Accumulation in liver and kidney was much less (1.93 ppm and 3.43 ppm respectively, at 1 ppm for 39 weeks) and insignificant levels were found in breast muscle (Woodham, Bond, Ahrens and Medley, 1975).

Dipping dairy cows once or twice daily with a 0.5% solution of toxaphene, produced the highest residue level in milk the day following the treatment (27-45 ppm on fat basis); a decrease to 5 ppm/fat basis was observed 19 days after suspension of dippings (Keating, 1978).

Levels of residues seen in surveys or monitorings on animal products are, of course, very variable. Very high levels have been detected recently in India, with maximum averages of 4.157 and 3.87 ppm in chicken fat, respectively for DDT and BHC (Kaphalia and Seth, 1981).

In western countries the levels for the single organochlorines are seldom in excess of 0.5 ppm. In USA, the average intake of DDT from feed, ranges, in poultry, from 5 to 25 ppb. Even at levels as high as 0.1 ppm of DDT in the feed, transfer of excessive residues in fat does not seem to occur, though a certain proportion of samples can show up to 1 ppm in adipose tissue. Fat, however is only a minor component of broiler carcass (Naber, 1977; Waldron and Naber, 1974).

In a survey performed at the Bologna slaughterhouse, muscle of adult cattle showed a constant presence of DDE, dieldrin and heptachlor epoxide; the highest mean value was total DDT (0.041 ppm/wet matter).

In perirenal fat there was a clear cut preponderance of total DDT (mean of 0.85 ppm, with some samples passing 2 ppm); DDE was the most represented single compound, followed by dieldrin. In the perirenal fat of 1 year old beef cattle residues were markedly smaller, owing to shorter exposure: the most important contamination resulted from dieldrin (0.163 ppm), possibly because of the corn silage feeding they had received (Crisetig et al., 1975).

Surveys carried out in Great Britain, Holland and Ireland between 1968 and 1971, showed a widespread contamination of beef by several compounds; however levels were mostly \leq 0.1 ppm on a fat basis. In France, meat showed detectable residues only of BHC and HCB, other compounds being present only in traces; however, in adipose tissue greater residues were detected, mainly BHC (0.167 ppm) and heptachlor epoxide (0.155 ppm) while DDT and its metabolites were still found only in traces (Richou-Bac and Cumont, 1971).

Organochlorine residues in foods of animal origin in Italy have been reviewed by Campnaini et al. (1980) with further information added by Madarena, Dazzi, Campanini and Maggi (1980). The residue levels are similar to those mentioned above, but the data lead to some further consideration: a certain degree of difference in meat contamination appears to be more species dependent, or due to unknown factors, than related to fat content; e.g. horse meat which had 1.96% of lipid content has been found to be more contaminated (average of 157.46 ppb/wet matter of total organochlorine) than other meats which were richer in fat, such as pork, which had 8.86% of lipid content and 79.46 ppm of

total organochlorine (Madarena et al., 1980). This relatively high contamination of horse meat has been partly confirmed by Palermo, Lo Muzio and Gentile (1978) who reported that all samples tested were contaminated with an average level of 58.6 ppb of total chlororganics on a wet basis. In ~~port~~, whereas contamination in adipose tissue is similar to that ~~seen~~ in cattle, meat contamination is higher and less dependent on fat content; moreover, it should be borne in mind that, at least in Italy, unlike beef fat, ~~port~~ fat is largely used as human food. K

Cooking does not reduce residues to any great extent, although frying can have some effect. A noteworthy effect of heat treatment is the conversion of DDT into DDD (Archer, 1976). Heavy apparent losses, caused by solubilisation of fat, can be seen after cooking, but, actually, the chlororganics are transferred to the broth or redistributed inside the food (Campanini et al., 1980). According to Naber (1977) between 66% and 80% of DDT, dieldrin and lindane can be recovered in drippings and broth. WHO (PRS, 1974) reported that 7.3 ppm of lindane in chicken fat were reduced to 5.5 ppm by frying and 3.9 ppm by boiling: unfortunately, the amounts in broth and drippings are not given. Canning shows some efficacy in reducing residues; beef fat samples containing 5 or 8 ppm of total DDT were heat treated either at 104°C for 137 minutes or at 126.7°C for 66 minutes: the 5 ppm residue decreased respectively by 19% and 21%, the 8 ppm by 8% and 12%: DDT showed major loss, DDE very slight loss but DDD increased. Further cooking in a microwave or conventional oven gave a further loss of 17% in the **canned** product residues (Earnsberger, Kilgore and Rogers, 1976). K

Trimming of fat and skimming of broth are crude but practical means of decreasing the content of residues (Archer, 1976; Campanini et al., 1980).

Pork products appear to be more contaminated than fresh pork, possibly because of the lower water and higher fat content (Campanini et al., 1980).

According to WHO (PRS, 1974) lindane was reduced by 34% in milk by spray drying but was not markedly affected by any other milk processing method. Archer (1976) reported that spray drying appeared to be the only method processing that markedly affected contamination, through removal of residues by codistillation. UHT or condensed milk showed only a slight reduction, while evaporated milk showed even an increase in residues, owing to a concentration effect, similar to that observed in butter and cheese. Ultra-violet irradiation reduced the residues in milk flowing over a surface cooler by 17%. With regard to cheese, certain bacteria (Brevibacterium sp. and Geotrichum sp. but not streptococci or micrococci) produced a decrease in DDT and DDE, while DDD remained unchanged or increased; these bacteria can be present in surface-ripened cheeses.

According to WHO (TRS, 1971-77) the (admissible daily intakes) ADI measured as ppm/body weight are as follows: dieldrin and aldrin 0.0001, endrin 0.0002, heptachlor 0.0005, HCB 0.0006, chlordane 0.001, DDT 0.005, lindane 0.01, methoxychlor 0.1. Besides ADI, WHO also recommends maximum residues for several items of the diet: e.g. in milk fat 0.15 ppm dieldrin or heptachlor; 0.1 ppm lindane; 0.5 ppm HCB and toxaphene; 1.25 ppm DDT.

2) Organic phosphorus compounds

a) General characteristics

Originally developed in the 1930s as potential agents in chemical warfare, the organophosphorus compounds are now the largest and most important group of pesticides. Clarke et al. (1981) reported that over 30,000 different compounds have been tested for insecticidal activity. The chemical characteristics of many compounds have been described in detail by Ware (1978) and by WHO (PRS, 1972-1976). They are mostly esters, amides or other simple derivatives of phosphoric and thiophosphoric acid and can be broadly divided into three classes: (a) aliphatic derivatives, such as dichlorvos, malathion, mevinphos, monocrotophos, trichlorfon. (b) Phenyl derivatives, including leptophos, ethyl and methyl parathion and many others. (c) Heterocyclic derivatives, as, e.g., chlorpyrifos and diazinon.

A very important problem, that can be just mentioned here, is selection of resistant strains of insects caused by insecticides. The widespread use of organophosphorus compounds was also due to the increased resistance to chlororganics, but resistant strains to many organophosphates are also developing (WHO TRS, 1980,b).

b) Persistence and metabolism

Although a few organophosphates can persist in the environment for more than a year, such as chlorfenvinphos and fonofos, a common feature of this pesticide class is their rapid hydrolysis outside and inside the organism and most are very unlikely to accumulate in biological systems (Edwards, 1975).

Many compounds are hydrophilic. They are rapidly absorbed, metabolised by the liver and excreted, mainly in the urine. Peak concentration in plasma is rapidly reached and then falls within a few hours, while traces can persist in liver and kidney for a few days only and appear briefly in milk (Gupta and Paul, 1977; Swan, Sauderhoff, Laskowski and Braun, 1977). A distinctive feature of trichlorfon is that it is converted, to a very small extent, in the body into the more toxic dichlorvos; however, both are hydrophilic and do not present any major residue problem, even at high dosages (Richou-Bac and Cumont, 1971; WHO PRS, 1973). A detailed appraisal of metabolism of individual compounds is given by WHO (PRS 1972-1976).

Several other compounds are worth greater concern because of their lipophilic behaviour; however, their concentrations in adipose tissue and lipid fractions of milk and body organs are markedly lower and less persistent than those of chlorinated hydrocarbons. Fenthion, for instance, is slowly metabolized and excreted and residues are present in body and milk fat and also, though in minimal amounts, in muscle, kidney and liver; they do not persist, however, more than 2 weeks in body tissue and one week in butterfat. Bromophos also, although markedly lipophilic,

does not seem to any great extent; its presence in the fat of lambs fell below the limit of detection within 22 days after treatment, while excretion in milk was very low and ceased within a few days (WHO PRS, 1972). Coumaphos showed traces, which disappeared within 29 days after treatment, in the fat of pigs and ruminants and in muscle of ruminants only, while butterfat was free from residues in approximately 60 hours (WHO PRS, 1973). Leptophos administered to hens peaked and disappeared in plasma within 72 hours, while in fat peak concentration was reached in 12 hours and decrease was quite slow, mainly in older birds, where, after feeding at 250 ppm, it persisted until 28 days (Konno and Kinebuchi, 1978).

Several other compounds, such as ethion and fenchlorphos (WHO PRS, 1973), bromophos ethyl and diazinon (WHO PRS, 1976) showed small residues only in adipose tissue and butterfat, which disappeared within a few weeks from the body and within a few days from milk. It is remarkable that poultry and pigs proved to be less prone to show traces of ethion and fenchlorphos than ruminants (WHO PRS, 1973).

Tetrachlorvinphos (Akhtar and Foster, 1981) and chlorpyrifos metabolites (Ivey, 1979; Ivey, Palmer and Hooten, 1978) presented low level residues in liver and kidney also.

c) Toxicity

Toxicity of organophosphorus compounds arises mostly from acetylcholinesterase inhibition, through phosphorylation of the receptor sites. Some of them act directly, others after having been metabolised in the body. A few organophosphates are more active against true cholinesterase and others against pseudo cholinesterase, but very few, if any, are really specific (Clarke et al., 1981). Organophosphorus toxicity can be modified by the action of chlorganics on liver microsomal enzymes (Tseng and Menzer, 1974).

Acetylcholinesterase inhibition does not necessarily result from intake of large doses, but also it is detectable, at least at biochemical level, after long term, low level feeding (WHO PRS, 1972-1976).

Short term trials have shown that several compounds inhibit aliesterase activity in plasma and liver at concentrations lower than those required for cholinesterase inhibition. The significance of this is still uncertain, but aliesterases are thought to play a role in drug metabolism and detoxifying systems (WHO TRS, 1973 and 1977).

The oral L_d50 in rats show a broad variation for the different compounds. It is, for instance, 3 mg/Kg body weight for parathion, 13 mg/Kg body weight for coumaphos, 27 mg/Kg body weight for ethion, 43 mg/Kg body weight for leptophos, 66 mg/Kg body weight for diazinon, 97 mg/Kg body weight for chlorpyrifos, 255 mg/Kg body weight for fenthion, 906 mg/Kg body weight for fenclorfos, 3750 mg/Kg body weight for bromophos (Ware, 1978).

WHO (PRS, 1972-1976) reported that chronic reproductive and/or hepatic impairment have been demonstrated, in long-term trials, for several compounds (e.g. carbofenthion, methidathion, trichlorfon), but at exposure levels extremely unlikely to arise from food intake. Furthermore, with regard to carbofenthion and methidathion, man seemed less susceptible than laboratory animals.

A toxic effect more worthy of concern is the so called delayed neurotoxicity. It is shared by the 5-phenylphosphonothioate esters (namely, in decreasing order of neurotoxicity: Cyanofenphos,)-ethyl O-p-nitrophenyl phenylphosphonothioate, desbromoleptophos, leptophos), by TOCP (tri-o-cresyl phosphate) and, at least experimentally, by others too (Abou Donia, 1979; WHO TRS, 1980,a). The neurotoxicity is characterized by ataxia and flaccid paresis, arising 8-14 days after recovery from acute intoxication, and demyelination of long nerve fibres. The basic mechanism appears to be depression of nerve protein synthesis. Severity is dose-related and only some species are sensitive, man possibly being the most (Harvey and Sharma, 1980; WHO TRS, 1980,a). Large doses are necessary (at least 10 mg/Kg body weight in hens for cyanofenphos) (Abou Donia, 1979), However WHO (TRS, 1973 and 1977) drew attention to the possible build up of low level residues to a toxic threshold. Actually, more than 40,000 human cases have been recorded. They arose mainly from the consumption of adulterated beverages in USA, 1930-31, and of adulterated olive oil in Morocco, 1959. In both incidents TOCP was incriminated. No human cases attributable to authorized use of pesticides or to residues in foods of animal origin have been so far recorded (WHO TRS, 1980,a).

d) Residues

Treatment of animals with organophosphates do not usually result in important residues, provided the instructions for use and the withdrawal times before slaughter and/or the utilisation of milk for human food are respected. Generally, even with lipophilic compounds, gross mishandling is required to produce consistent amounts in animal products.

Residue levels of chlorpyrifos in ruminants and pigs, following different application methods, have been studied by several authors (Ivey, 1979; Ivey et al., 1978; Ivey and Palmer, 1979; Ivey and Palmer, 1981; WHO PRS, 1973). The compound was present mainly in fat, the highest residue (1.6 ppm) being seen in cattle after a series of multiple dippings, and the longest persistence (6 weeks, both for the compound in body fat and for the pyridinol metabolite in liver and kidney) being in sheep after pour-on treatment.

Administration, by different application methods, of coumaphos to cattle always led to residues (before) 1 ppm in muscle and fat. In pigs, after 6 applications of 0.5% spray at 14 days intervals, residues in meat disappeared, and those in fat fell below 0.15 ppm, within 8-29 days. Sheep, after an analogue series of sprays at 0.25%, showed 1.7 ppm in fat after 8 days, falling to 0.1 ppm within 29 days (WHO PRS, 1973).

According to Shanks (1975), pour-on applications of 7.5-30 ppm/body weight of fenchlorphos in cows produced a peak of 1.8-2.1 ppm in butterfat after 3 days, reducing to 0.01 ppm after 28 days.

Ivey et al., (1976) reported that 0.5% spraying with fenchlorphos in hens produced a peak of 1.207 ppm in skin and 0.825 ppm in fat after 1 day, and traces were detected in skin after 28 days, but failed to show accumulation after a second treatment.

After dipping cattle with dioxathion, maximum residues in fat were reached within 2-4 days after treatment, with a half-life, similar to ethion or chlorpyrifos, of about 16 days (Palmer, Dingle and O'Neill, 1977). Residues in milk fat persisted for 3 days and ranged from 0.25 to 0.8 ppm (Keating, 1978).

Famphur administered to cows as pour-on application at 23 ppm/body weight, produced the highest residues in milk after one day (0.237 ppm/whole matter) and fell to 0.08 ppm the third day; 76% of the residues was concentrated in the butterfat. In the body, famphur residues were markedly higher in muscle than in liver or kidney (0.53 ppm and 0.05 ppm, respectively, 7 days after pour-on treatment at 45 ppm/body weight) (Annand, Dingle, Heath and Palmer, 1976).

Results similar to those above mentioned have been reported for bromophos, bromophos ethyl, chlorfenninphos, fenthion and others (WHO PRS, 1972-1976). It should be kept in mind, moreover, that in general all cows in a herd will not be treated simultaneously, so that residues in bulk milk should be subjected to a certain degree of dilution (WHO PRS, 1976).

Organophosphates have little potential as accidental or environmental contaminants, as several data appear to suggest.

In surveys carried out in USA in 1964-1966, only traces of diazinon, fenchlorphos and parathion were found in meat, and

traces of malathion in fat (Richou-Bac and Cumont, 1971).

Begliomini and Fravolini (1973), in a feedstuffs survey, found, just in a tiny proportion of corn and barley meal, small residues of methyl-parathion only.

According to american authors (Leuck, Johnson, Bowman, Knox and Berota, 1977), corn treated with fenitrothion (31b/acre) and fed to cows for 8 weeks led to residues of 1-5 ppb in milk.

Cows fed 20 ppm of ethion for 30 days showed a peak concentration in milk of 34 ppb/whole matter after 4 days, and no residues were detected after withdrawal from the ration (WHO PRS, 1973). In turkeys confined in soils treated with ethion (44 Kg/ha) residues peaked within a week and were 0.295 ppm and 0.666 ppm in fat and skin respectively (Ivey, Kunz and Mann, 1975).

According to WHO (PRS, 1976), 10 ppm/feed of chlorpyrifos administered to hens for 30 days are unlikely to give traces exceeding 0.1 ppm in fat and 1 ppm of the pyridinol metabolite in liver and kidney (WHO PRS, 1976). Turkeys confined in pens sprayed with chlorpyrifos, showed peak concentration of 0.152 ppm in skin and 0.055 in fat after one week; the pyridinol metabolite was present in skin, kidney, liver at concentrations of 0.003 - 0.165 ppm (Mann, Ivey, Kunz and Hogan, 1973).

Spraying of cow housings with tetrachlorvinphos resulted in residues below 0.01 ppm in milk, which persisted for 5 days (Milhaud, Bechade and Pinault, 1975).

Heat treatment led to considerable breaking down of organophosphorus pesticides. WHO (PRS, 1972) reported that boiling for 1 hour caused the disappearance of up to 10 ppm of

trichlorfon in sheep meat, while in beef meat residues of 0.53 ppm of trichlorfon were reduced to 0.1 ppm, in the broth also.

Other compounds showed similar reductions, although the phenomenon has been more studied in the vegetables (WHO PRS 1972-1976).

Prolonged cold storage also has some effect on residues; bovine livers and kidneys contaminated by fenthion showed after 6 weeks at -18°C a decrease by 80% and by 20% respectively, while losses were slight for brains, hearts, fat and steaks. In another trial, however, fenthion decreased in liver just by 20% after 12-26 weeks at -18° - -23°C (WHO PRS, 1972).

In milk organophosphates tend to be associated with the lipid fraction and to concentrate in butter. Gaiduskova (1974) has evaluated the proportions passing from cream to butter for the following compounds: Malathion (65-77%), fenitrothion (62-67%) and dichlorvos (53-60.5%). In a previous study, the author evaluated the proportions present in fat phase of milk after centrifugation as 90% for malathion, 84% for fenitrothion, 40% for dichlorvos. She also reported that after storage of the butter at 20°C and -25°C for 28 days, losses on butter residues were respectively 43% and 32.5% for malathion, 26% and 13% for fenitrothion, 32% and 21.9% for dichlorvos. The author suggested that PH also could influence the rate of break-down; butter made from sour cream would be expected to have greater losses, owing to the action of lactobacilli. She concluded that losses in butter were significant, but that some protective action from fat phase was demonstrated.

According to WHO (TRS, 1971-77), ADIs measured as ppm/body weight are 0.0005 for fenthion and coumaphos, 0.001 for leptophos, 0.0015 for chlorpyrifos, 0.004 for dichlorvos, 0.005 for cyanofenphos, ethion, fenitrothion, trichlorfon, 0.006 for bromophos and are relatively high for fenchlorphos (0.01) and malathion (0.02). Examples of maximum recommended residues in milk are 0.1 ppm for chlorpyrifos and fenitrothion in milk fat and 0.02 ppm and 0.05 ppm on whole matter basis for bromophos and ethion, respectively.

3) Sampling and monitoring

Food animals exhibiting acute signs of intoxications are very unlikely to be normally slaughtered for human consumption. Chronically contaminated animals do not show pathognomic signs (Clarke et al., 1981; Grossklaus, 1978), although disruption of porphyrin metabolism, resulting in ultra-violet fluorescence, has been noted in sheep under natural conditions in contact with lindane (Nichol et al., 1981) or in swine after experimental feeding with HCB (Hansen et al., 1977). Animals bearing high level residues easily pass through routine meat inspection procedures; inspection programmes must rely on monitoring of collected samples.

Emphasis has been put, by several authors, on in vivo sampling methods for large animals. Early detection of unacceptable residue levels could allow salvaging of a valuable carcass by withdrawal of the slaughter.

Blunt and Saunders (1978) described a bioptic technique for scrotal fat in cattle and recommended it as an aid to decide when animals should be sent to slaughter.

Blood levels in DDT-fed cattle were indicative of dosages (Whiting et al., 1973) and an approximate 1000-fold ratio was seen between ppb in blood and ppm in fat of naturally contaminated animals (Campos, Gutierrez and Olszyna Marzys, 1979); a 1000-fold and a 500-fold ratios were reported respectively in sheep and pigs fed HCB (Avrahami and Steele, 1972; Tonkelaar et al., 1978): blood sampling, therefore, can be used to measure in vivo previous

exposure to, at least, some persistent compounds. The same may be said for placenta, where total DDT residues, though very small (5-17 ppb) were correlated with those in the fat of the newborn calf; correlation was not linear but fairly accurate for DDE (by far the highest residue), less precise for DDT and DDD (Whiting et al., 1972,a).

Milk sampling for meat inspection purposes is easier than body fat biopsy: the constancy of ratios of milk fat to body fat residues, for the persistent chlororganics, makes it an efficient means of selecting animals with body fat levels above the guidelines (Fries, 1977); on the other hand, testing of a biopsy sample of tailhead fat in dry cows could be utilised to forecast residues in milk during the coming lactation (Stull, Brown and Whiting, 1971).

With regard to methods of post-mortem sampling, it has been noted that kidney and liver contents of total DDT were correlated with levels of exposure and residues in adipose tissue, while muscle content was not (Whiting et al., 1972,a; Whiting et al., 1973). Heptachlor epoxide distribution in broilers was not uniform, according to Wagstaff et al. (1980), so that sampling of fat can result in an underestimation of residues in the lipid fractions of liver and muscle; however, this possible bias should not cause undue concern, because lipids are a very small proportion of these tissues (in liver 1.5%). On the other hand, Kan and Jonker-den Rooyen (1978,b) found in hens a very high correlation between chlororganic residues in abdominal fat and in lipid fractions of liver and breast and thigh muscle.

The above reported considerations about sampling methods can be valid for organophosphorus compounds also, owing to the fact that the most important organophosphates from the point of view of food hygiene are the lipophilic ones. Unfortunately, sampling problems for these compounds have not so far been described.

It is not the purpose of this thesis to deal with chemical analytical methods: a comparison of different techniques for chlorinated hydrocarbons is presented by Smart, Hill and Roughan (1974); analytical methods for many organophosphates are reviewed by WHO (PRS, 1972-1976).

According to Edwards (1975), monitoring by checking spot samples of foods and feedstuff is the most satisfactory way of surveillance. This may involve analysis of a big number of samples; for instance, about 50,000 samples of harvested crops have been analyzed annually in USA. Standardization of sampling and analytical procedures is required, as adequate information on sensitivity and specificity of analytical methods. With an admissible level of 10 ppm, an error of 10% can be acceptable, whereas with very small residues tolerances, the potential errors increase greatly so that a possible error of 100% would be expected and acceptable with a tolerance limit of 0.001 ppm.

Finally, Spencer (1971), pointing out the aims of pollution control, stated that most contaminated areas (i.e. where capacity of the biological substrate to fully metabolize residues is overcome) will be local and monitoring should be able to pinpoint these trouble spots.

4) Prevention

Apart from monitoring, Kaferstein and Lorenz (1978) and Matyas (1978) suggested several steps to prevent or minimize occurrence of pesticide residues in human foods. Administration of drugs to animals should be strictly professionally justified and priority should be given to the use and development of compounds less likely to give persistent residues, of course bearing in mind the economical considerations. The local veterinary services should be notified of pesticide treatments of any vegetable intended for fodder, as of any feedstuffs under storage; they should also check from time to time whether all requirements for safe use are fulfilled. Withdrawal period after drug application should be carefully planned and strictly respected as, when possible, the interval between last application to feed and use, to avoid toxic effects or undesirable residues; collaboration from field veterinarians is essential and should be encouraged. WHO (TRS, 1972) also suggested that dairy animals should be treated during dry period or, when this is not possible, all the animals should not be treated at the same time.

With regard to environmental contamination, establishment of new animal productions in areas under continuous heavy exposure should be discouraged. Existing animal industries showing residues continually above acceptable levels should be removed or remodelled or, at least, should be supplied with feed from clean areas (Matyas, 1978). Edwards (1975) pointed out that a rational use of pesticides, avoiding excessive spreading, was very important in minimizing environmental pollution.

According to informations available on toxicity and persistence, maximum residue levels should be defined for the various compounds, and applied also to imported foods and feedstuffs. Tolerances set on organochlorines by different countries have been reviewed by Edwards (1975); usually, very wide safety margins, often up to 100 times, are used to account for the appearance of any one pesticide in several different diet items and/or for interactions with other chemicals and/or for potential greater sensitivity of children or older people. The tolerances suggested by WHO are generally accepted as useful guidelines.

Since the late '60s, many countries have banned or more or less severely restricted the use of several persistent chlororganics (e.g. DDT, aldrin, dieldrin, endrin, heptachlor) (Edwards, 1975). Ware, Estes, Buck and Martello (1978) noted that, after 8 years of DDT moratorium in Arizona, residues in beef fat decreased by 62%.

With regard to animals already contaminated, several methods have been suggested for decontaminating them in an economically reasonable time. These methods include administration of activated charcoal, tannic acid or phenobarbital, but their efficiency seems very limited. Injections of vitamin A,D,E markedly reduced half-life of dieldrin residues in beef cattle (Cook and Wilson, 1971). A more simple method of decontaminating animals is their transfer into a clean environment. This method may be preferable in poorer countries, instead of rejecting a useful carcass (Campos *et al.*, 1979). On the other hand, the time needed to reach acceptable levels could be quite long.

According to Whiting, Stull and Brown (1972), lactating cows, whose milk contained 1.79 ppm of total DDT on fat basis required 8-10 months on a low-pollution pasture to fall to or below 0.21 ppm, with an average half-life of residues of 2 months. Dry contaminated cows, which began lactation after transfer, showed half-lives of residues of about 4 months.

5) Hazards for the consumers

While organophosphorus compounds do not appear to cause particular problems if used in accordance with regulations and good agricultural practice, chlorinated hydrocarbons are of more concern.

Most chlororganics are now being phased out, however they persist in areas of previous usage. A great proportion (about 85-90%) of human intake of chlororganics is assumed to be due to food (Edwards, 1975). Up to 50% of all ingested organochlorines are derived from foods of animal origin. DDT intake by USA population was considered to be mainly due to meat, fowl and fish (all accounting for 0.024 mg/day) followed by milk and dairy products (0.01 mg/day). On the other hand, intake of dieldrin/aldrin derived mainly from milk (0.0002 mg/day), then from meat. Heptachlor epoxide intake from meat was approximately equal to that from milk, each accounting for about 0.0001 mg/day, while lindane alone showed an intake from vegetables greater than from foods of animal origin. However, intake of chlorinated hydrocarbons, other than DDT, was so low that meat could be regarded as the most important alimentary source of organochlorines in USA (Grossklau, 1978).

Broad variations between different countries, often unexplained, may be connected with different agricultural practices. In Great Britain, milk and dairy products have been reported to be the least contaminated item of the diet, while in USA they occupy the second highest position (Edwards, 1975).

Comparisons between potential daily intakes through diet and ADIs were carried out by WHO (TRS, 1972, 1976, 1977) in 5 countries with different socio-economical characteristics. It was shown that, for most organophosphates, there was no possibility, even theoretical, of exceeding ADI; a few compounds (among them leptophos and parathion methyl) showed some possibilities of exceeding the threshold in one or more countries; for three compounds (namely metamidophos, pyrimiphon-methyl and, mainly, fenthion) the possibilities were greater. There is in effect some evidence that, as organophosphates gradually substitute chlororganics, their residues in human food tend to increase (Edwards, 1975). On the other hand, comparison between ADI and potential intake of chlororganics showed that chlordane, endrin, HCB and heptachlor were borderline, while DDT and dieldrin could reach unacceptable levels. WHO warned, however, that the above data should not be overevaluated, owing to the very generous, rather than overstringent, calculations both of ADI and of potential intake.

Total diet studies carried out in U.K. (Edwards, 1975) and in Italy (Campanini et al., 1980) revealed that residues of DDT and its metabolites exceeded the total of the other compounds and that residues were well below unacceptable levels. In Italy, dieldrin was the nearest to the limit, its intake being, as average, 46% of ADI; bovine liver was the item showing the highest dieldrin contamination, while the most important foods for annual dieldrin intake were milk, milk products and potatoes.

With regard to levels of chlorinated pesticides stored in human fat, Campanini et al. (1980) and Edwards (1975) reported that the highest levels were seen in India, in 1964, with 28 ppm total DDT, 11.6 ppm DDE, 1.7 ppm γ BHC and 0.03 ppm dieldrin/aldrin in vegetarians and 12 ppm, 6.4 ppm, 0.9 ppm and 0.06 ppm, respectively, in meat eaters. High values were found also in Israel in 1965-1966 (18.1 ppm total DDT and 9.9 ppm DDE), Italy in 1967 (8.52 ppm and 7.5 ppm respectively) and USA in 1967-1970, where residues ranged from 4.5 to 10 ppm for total DDT and 3.8 to 7.2 ppm for DDE, mean amounts in people from southern states being about 2-fold those of people from the north. Countries of northern Europe showed smaller residues and Australia even less (in 1967: total DDT 1.7 ppm, DDE 0.93 ppm). It was noteworthy that, with very few exceptions, chlorinated pesticides other than DDT and its metabolites averaged well below 1 ppm in all countries. No endrin was reported in any of the surveys.

Several studies have been performed on chlororganic concentrations in milk of not-exposed women of several countries. Residues were always and those of DDT could average about 0.2 mg/litre (Edwards, 1975).

To date, this continuous, sometimes heavy, exposure could not be associated with any pathological condition in man. No significant differences have been found between chlororganic levels in bioptic and autoptic human samples. Workers in a dieldrin factory showed residues in body fat 9 times greater than those of the general population, but there was no significant correlation between sickness and residue levels or between hours of exposure

and residual amounts. It was noteworthy that, though endrin was also manufactured in the factory, no residues were found in the samples. Lack of relationships between DDT concentrations and incidence of cancer or of other **chronic** conditions has been previously mentioned (Edwards, 1975).

However Grossklaus (1978) points out that it may be very difficult to spot and characterize the chronic danger from the continuous ingestion of small quantities of certain compounds having one or more biological targets (e.g. the liver) and, therefore, the population might be exposed to a high risk for this very reason. Moreover, the presence of residues in foodstuffs, revealed by surveillance of the importing countries, can create considerable economical problems to food-exporting countries (Richou-Bac and Cumont, 1971).

DISCUSSION

Chlorinated hydrocarbons and organic phosphorus compounds, notwithstanding their different pharmacological and toxicological characteristics, can be considered together from a public health point of view. Both can be present in food as residues originating either from the use and misuse of pesticides in veterinary therapeutic practice or from environmental pollution. The latter is preeminent for chlororganics as they can be present in ecosystems, and also in animal products, even years after having been banned, owing to their great resistance and lipophilic properties.

Organochlorine accumulation occurs in adipose tissue and lipid fractions of several organs (mainly liver and kidney) and milk, whilst it occurs to a smaller extent in the lipid fraction of muscle. All compounds show a fairly constant ratio between concentration in tissues or milk fat in each species and intake; the ratio values are correlated both with persistence and cumulative potential of the chemical and with the metabolic characteristics of the species. Generally, ratio values are smaller for chlordane, DDD, lindane, toxaphene and greater for } BHC, DDE, dieldrin, HCB, heptachlor epoxide. A very remarkable feature is that accumulation does not appear to proceed beyond an upper limit: this is of great importance, from the food hygiene point of view, because it implies that residues do not build up indefinitely, even with a life-long exposure. Differences in accumulation patterns exist among compounds as well as among species; even fat depots appear to be contaminated to a different extent.

The above considerations entail that each compound in each animal species has a different potential of getting to the consumer in a significant amount and they also can cause some bias in sampling methods (Wagstaff et al., 1980). The residues of most compounds show a very prolonged half-life and they may require several weeks or months to show any significant decrease in animal tissues or milk.

With regard to organophosphorus pesticides, many of them are hydrophilic and so rapidly metabolised and excreted that, if gross mishandling is avoided, they should not be present in amounts greater than traces in animal products and, therefore, they are generally of little importance in food hygiene.

After treatment of farm animals with lipophilic organophosphates, small residues of moderate persistence are present, mainly in adipose tissue and milk fat; a period of a few weeks is required for their disappearance, or substantial reduction, in tissues, while in milk their persistence is much shorter. However, their cumulative properties are far below those of the chlororganics and their potential as environmental pollutants is little. Organophosphorus residues in human food are, therefore, generally associated with faults in controlling their use; however, they are so widespread, that they may make a small, but possibly increasing, contribution to environmental pollution.

From a toxicological point of view, a quite broad range of chronic disruptive effects on several metabolic systems are recognized in animals. Targets of organochlorine action are the liver, endocrine glands and reproductive apparatus, while organophosphates can cause subclinical acetyl-cholinesterase inhibition.

Both classes of compounds also may be responsible for alterations of enzymatic response to foreign substances, which might lead to interactions with other toxic compounds.

Remarkably, oncogenic effect is not shown by any compound. The WHO did not consider that the increased incidence of hepatomas in mice (but not in any other species) administered with high doses of DDT or lindane would constitute a finding of some concern for public health.

A small group of organophosphates has been responsible for disease in consumers, the so called delayed neurotoxicity syndrome, but, as far as it is known, this effect has never arisen from chronic exposure to low level residues, as are likely to be present in meat or milk. To date, no pathological effects in humans are known to be caused by pesticide residues in food of animal origin.

Another reason for not overemphasizing the importance of the presence of pesticide residues is that those seen in experimental studies are generally higher than those found in natural situations in western countries; elsewhere, e.g. in India, the situation can be different. In most instances, residues detected in surveys are, on average, below 1 ppm, owing to a very low and/or discontinuous intake of pesticides by farm animals. Non-persistent compounds will be usually found in foods only in rare cases of misuse.

The almost exclusive lipophilic pattern of accumulation of chlororganics and of the more persistent organophosphates, entails that their residues will be diluted or dispersed to a certain

degree if the whole food item (and not just its lipid fraction) is considered. On the other hand, residues will be concentrated in products with a high lipid content (e.g. butter, cheese, pork products) simply because of the increase of the potentially contaminated fraction.

Organochlorines are fairly resistant to many methods of food processing and a great proportion of the initial residue can pass from the raw material to the final product. Organophosphates are markedly less stable, but, when residues are present, a significant proportion of them can pass through the processing and reach the consumer.

Chlorinated hydrocarbons and organophosphorus compounds are potentially harmful chemicals; their presence in human foods is undesirable and should be always monitored and not permitted above certain levels. As residues usually escape detection by routine meat inspection or milk control procedures, contamination should be monitored by chemical analysis of collected samples. Of course, standardisation and reliability of sampling and analytical methods must be ensured.

Whenever possible, large animals should be sampled in vivo, by means of fat biopsy or blood or milk sampling. It would be preferable to perform this sampling on the farm, some time before slaughter. It would be more economically sensible, instead of rejecting valuable carcasses, to decontaminate the animals by withdrawing them from slaughter for a time sufficient for the residues to fall to an acceptable level. The time required for this purpose can be lengthy and economical convenience should be always borne in mind; it would be economically infeasible to

withdraw from slaughter fast-grown beef cattle or pigs, if tested shortly before slaughter, and even more difficult (not to say impossible) to withdraw a flock of broilers. More generally, if just a few samples will reveal unacceptable levels, the withdrawal measures will be of minimal social and economical usefulness and many farmers will disagree with them. However, if a large proportion of animals will result contaminated, these measures will be very likely to be both welcome and economically convenient.

Biopptic samples in dry dairy animals can be used also to forecast possible future milk contamination. Withdrawal of milk from human consumption or its utilisation as spray dried milk and transfer of the animals into a clean environment can be applied to dairy farms, but with the practical problems already mentioned.

These problems are much less serious for organophosphorus residues, whose depletion times are much quicker than those of chlororganics and which are less resistant to food processing. However their presence also should be monitored as well as that of every potentially toxic compound used on, or likely to come in contact with farm animals.

It is completely unrealistic to think that every live animal, every carcass or every bulk sample of milk could be sampled and analysed. A more realistic approach to this problem is a programme of monitoring of random samples. Routinary examination of animals and animal products might be required only in some very limited areas, or in some farms, where contamination at high levels is particularly frequent. However the high costs of a regular

monitoring of random samples (or even of a programme of surveys on certain animal products or areas) would have to be born in mind. The personnel involved, the number of samples necessary to be representative, the chemical laboratories would make this method of control very expensive. The costs would be, at least in part, compensated if data furnished by monitoring were utilised primarily for "epidemiological" purposes (i.e. to look at the quantitative and qualitative features of pollution and, where tracing-back facilities permit, its geographical distribution) rather than for judging the fitness for human consumption of the individual samples. Residues might be a useful indicator for monitoring trends of general environmental pollution. Control programmes could furnish data to establish or modify, if needed, preventive actions and to evaluate their feasibility; they could be used to intensify or decrease further surveillance, to focus attention to some particular areas.

It should be stressed that a policy of control and prevention of pesticide residue occurrence in food should not be fully developed when other more important veterinary public health problems are still out of control. This policy should be undertaken and expanded mainly, or only, in developed countries, where a more or less efficient control of more pressing problems (e.g. certain zoonotical conditions) has already been achieved. However, some kind of surveillance of residue occurrence and distribution should always be performed, as it may constitute a basis of information for future monitoring and control programmes.

Kaferstein and Lorenz (1978) and Matyas (1978) suggested several preventive steps that could be undertaken in an ideal situation and that have already been mentioned. Some of them are, at present, of more theoretical than practical interest but others are really important. The firmly established control over pesticide use is the main one and it would be more efficient if veterinary practitioners were made more aware of residue problems and became involved in the application of preventive policies. Another essential point is the development of efficient compounds devoid of major public health risks.

Presence of chlororganic residues in human body fat and even in human milk is now well known, and average intakes through diet have been evaluated in some countries. However, appraisal of the existence and of the importance of possible effects arising from the exposure to food residues is very difficult. The toxic action (S) could be very subtle and could appear insidiously and also interactions with many other factors could be possible. Difficulties in detecting effects of compounds of known toxicity might lead to a high, unrecognized risk in the exposed population. To date, no meaningful relationship has been found between human chronic diseases and chronic exposure to persistent pesticides. Control of pesticide contamination, however, is still justified by both the danger of ecological damage and the possible exposure of the population to a risk difficult to determine and unknown in its real dimension.

REFERENCES

- Abou-Donia, M.B. (1979) Delayed neurotoxicity of phenylphosphonothioate esters. Science, USA, 205, 713-715
- Ahrens, F.A., Dyer, D.C., Lloyd, W.E. (1980). Mirex kinetics in chickens. J. Toxicol environ Health, 6, 835-842
- Akhtar, M.H. & Foster, T.S. (1981). Tetrachlorvinphos metabolism in laying hens. J. agric. Ed chem, 29, 766-771
- Annand, A.M., Dingle, J.H.P., Heath, A.B. & Palmer, W.A. (1976). Residues of famphur in bovine tissues and milk following its application as a pour-on insecticide. Aust. J. exp. Agric. Anim. Husb., 16, 82-87
- Archer, T.E. (1976). Stability of DDT in food and feeds, transformation in cooking and food processing, removal during food and feed processing. Residue Rev., 61, 29-36
- Arthur, R.D., Cain, J.D. & Barrentini, B.F. (1975). The effect of atmospheric levels of pesticides on pesticide residues in rabbit adipose tissue and blood sera. Bull. environ. Contam. Toxicol., 14, 760-764
- Avrahami, M. & Steele, R.T. (1972). Hexachlorobenzene. I. Accumulation and elimination of HCB in sheep after oral dosing. II. Residues in laying pullets fed HCB in their diet and the effect on egg production, egg hatchability and on chickens. III. The effects of feeding HCB to growing chickens. N.Z. J. agric. Res., 15, 476-494
- Begliomini, A. & Fravolini, A. (1973). Residui di insetticidi nei manofimi. IV. Idrocarburi clorurati ed esteri fosforici nelle farine di posee, orzo, granturco e soia diprovenienza estera. Archo vet. ital., 24, 183-190
- Blunt, C.G. & Saunders, P.J. (1978). The distribution, live sampling for and decline of DDT in steers. Aust. J. exp. Agric. Anim. Husb., 18, 335-339
- Bond, C.A., Woodham, D.W., Ahrens, E.H. & Medley, J.G. (1975). The cumulation and disappearance of mirex residues. II. In milk and tissues of cows fed two concentrations of the insecticide in their diet. Bull. env. Cont. Tox., 14, 25-31
- Campanini, G., Maggi, E. & Artioli, D. (1980). Present situation of organochlorine pesticide residues in food of animal origin in Italy. Wld. Rev. Nutr. Diet., 35, 129-171
- Campos, M. de., Gutierrez, G.E. & Olszyna Marzys, A.E. (1979). Correlation between total DDT residues in blood and fat of beef animals. J. Food Prot., 42, 948-949
- Clarke, M.L., Harvey, D.G. & Humphreys, D.J. (1981). Veterinary toxicology, Bailliere Tindall, London

- Cook, R.M. & Wilson, K.A. (1971). Removal of pesticide residues from dairy cattle. J. Dairy Sci., 54, 712-717
- Copeland, M.F. & Cranmer, M.F. (1974). Effects of o, p'-DDT on the adrenal gland and hepatic microsomal enzyme system in the beagle dog. Toxic. appl. Pharmac., 27, 1-10
- Crisetig, G., Mora, A. & Virrani, R. (1975). Residues of persistent organochlorine compounds in the tissues of cattle. Folia Vet. Lat., 5, 1-26
- Driver, D., Brewer, R.N. & Cottier, G.J. (1976). Pesticide residues in eggs and chicks from laying hens fed low levels of several chlorinated hydrocarbon pesticides. Poult. Sci., 55, 1544-1549
- Earningsberger, A.P., Kilgore, L.T. & Rogers, R.W. Degradation of 1,1,1-trichloro -2,2-bis (p-chlorophenyl) ethane (DDT) in beef by canning and cooking. J. of agric. Fd Chem., 24, 577-678
- Edwards, C.A. (1975). Persistent pesticides in the environment 2nd edition. CRC press, Cleveland
- Fishwick, F.B., Hill, E.G., Rutter, I. & Warre, P.R. (1980). Gamma HCH in eggs and poultry arising from exposure to thermal vaporisers. Pestic. Sci., 11, 633-642
- Foster, T.S., Morley, H.V., Purkayastha, R., Greenhalgh, R. & Hunt, J.R. (1972). Residues in eggs and tissues of hens fed a ration containing low levels of pesticides with or without charcoal. J. econ. Ent., 65, 982-988
- Fries, G.F. (1977). The Kinetics of halogenated hydrocarbon retention and elimination in dairy cattle. In Fate of pesticides in large animals (ed. G.W. Ivie and H.W. Dorough), pp. 159-173, Academic Press, New York
- Fries, G.F. & Marrow, G.S. (1977). Distribution of Hexachlorobenzene residues in beef steers. J. Anim. Sci., 45, 1160-1165
- Gajduskova, V. (1974). Dynamics of organophosphorus pesticides residues in butter during manufacture and storage. Milchwissenschaft, 29, 278-280
- Gilbert, W.S., Singh, G. & McIndoe, R.N. (1976). DDT residues in poultry from rice hulls used as poultry litter. Austr. J. exp. Agric. Anim. Husb., 16, 701-708
- Grossklaus, D. (1978). The occurrence and importance of residues in meat and their evaluation within official meat inspection. Ann. Ist. Super. Sanita, 14, 319-334
- Gupta, P.K. & Paul, B.S. (1977). Biological fate of malathion in Gallus domesticus (Desi poultry birds). Toxicology, 7, 169-177

- Hansen, L.G., Dorn, S.B. & Beamer, P.D. (1979). Residues and effects from feeding high concentrations of hexachlorobenzene to broiler cockerels. Poult. Sci., 58, 81-86
- Hansen, L.G., Simon, J., Dorn, S.B. & Teske, R.H. (1979). Hexachlorobenzene distribution in tissues of swine. Toxic. appl. Pharmac., 45, 541-547
- Hansen, L.G., Wilson, D.W., Byerly, C.S., Sundlof, S.F. & Dorn, S.B. (1977). Effects and residues of dietary Hexachlorobenzene in growing swine. J. Toxic. environ. Health., 2, 557-567
- Harvey, M.J. & Sharma, R.P. (1980). Organophosphate cytotoxicity: the effects on protein metabolism in cultured neuroblastoma cells. J. environ. Pathol. Toxicol., 3, 423-436
- Ivey, M.C. (1979). Chlorpyrifos and 3,5,6-trichloro-2-pyridinol: residues in the body tissues of cattle wearing chlorpyrifos-impregnated plastic ear tags. J. econ. Ent., 72, 909-911
- Ivey, M.C., Kunz, S.E. & Mann, H.D. (1975). Ethion, Ethion monooxon and ethion dioxon: residues in the body tissues of turkeys confined in pens on treated soil. J. econ. Ent., 68, 353-354
- Ivey, M.C., Mann, H.D. & Wright, F.S. (1976). Ronnel and its oxygen analogue: residues in body tissues and eggs of caged layers. J. econ. Ent., 69, 744-746
- Ivey, M.C., & Palmer, J.S. (1979). Chlorpyrifos and 3,5,6-trichloro-2-pyridinol: residues in body tissues of swine treated with chlorpyrifos for hog louse and itch mite control. J. econ. Ent., 72, 837-838
- Ivey, M.C. & Palmer, J.S. (1981). Chlorpyrifos and 3,5,6-trichloro-2-pyridinol: residues in body tissues of sheep treated with chlorpyrifos for sheep Keol control. J. econ. Ent., 74, 136-137
- Ivey, M.C., Palmer, J.S. & Hooten, E.C. (1978). Chlorpyrifos and 3,5,6-trichloro-2-pyridinol: residues in body tissues of cattle wearing chlorpyrifos-impregnated plastic ear bands. J. econ. Ent. 71, 697-700
- Kaferstein, F.K. & Lorenz, H. (1978). Legislative and technical measures at the production level aiming at the prevention or reduction of chemical and other residues in food of animal origin. Ann. 1st Super. Sanita., 14, 335-344
- Kan, C.A. (1978). Accumulation of organochlorine pesticides in poultry: a review. J. agric. Fd Chem., 26, 1051-1055

- Kan, C.A. & Jonker-den Rooyen, J.C. (1978). Accumulation and depletion of some organochlorine pesticides in broilers breeder hens during second laying cycle. J. agric. Fd Chem., 26, 465-470
- Kan, C.A. & Jonker-den Rooyen, J.C. (1978). Accumulation and depletion of some organochlorine pesticides in high-producing laying hens. J. agric. Fd Chem., 26, 935-940
- Kan, C.A., Jonker-den Rooyeh, J.C., Tuinstra, L.G.M., Roos, A.H. & Traag, W. (1978). Possible influence of sex and embryonic content on accumulation of some organochlorine pesticides in broilers. J. agric. Fd Chem., 26, 618-621
- Kaphalia, B.S. & Seth, T.D. (1981). DDT and BHC residues in some body tissues of goats, buffalo and chickens, Lucknow, India. Pestic. monitor. J., 15, 103-106
- Keating, M.I. (1978). Excretion of toxaphene and dioxathion in the milk of dairy cows. Bull. anim. Health Prod. Afr., 26, 285-292
- Konno, N. & Kinebuchi, H. (1978). Residues of phosvel (leptophos) in plasma and in adipose tissue of hens after single oral administration. Toxic. appl. Pharmac., 45, 541-547
- Latimer, J.W. & Siegel, H.S. (1977). DDT and metabolites accumulation in adrenal, liver and brain of broiler chickens. Poult. Sci., 56, 1622-1626
- Leuck, D.B., Johnson, J.C. jr., Bowman, M.C., Knox, F.E. & Beroze, M. (1971). Fenitrothion residues in corn silage and their effects on dairy cows. J. econ. Ent., 64, 1394-1399
- McBlain, W.A., Lewin, V. & Wolfe, F.H. (1974). Limited accumulation of DDT in eggs, fat and livers of Japanese quail. Poult. Sci., 53, 84-88
- Madarena, G., Dazzi, G., Campanini, G. & Maggi, E. (1980). Organochlorine residues in meat of various species. Meat science, 4, 157-166
- Mann, H.D., Ivey, M.C., Kunz, S.E. & Hogan, B.F. (1973). Chlorpyrifos, its oxygen analogue and 3,5,6-trichloro-2-pyridinol: residues in the body tissues of turkeys confined in pens on treated soil. J. econ. Ent., 66, 715-717
- Matyas, Z. (1978). Veterinary public health activities aimed at prevention of intoxications by chemical residues in foods of animal origin. Suggestions for inspection programmes. Ann. 1st. Super.Sanita, 14, 301-314
- Milhaud, G., Bechade, A. & Pinault, L. (1975). Contamination du lait par des residues de bromophos ou de tetrachlorvinphos utilises pour la desinsectisation des etables. Lait, 55, 163-170.

- Moore, S., Bruce, W.N., Kuhlman, D.E. & Randell, R. (1973). A study of the sources of insecticides residues in milk on dairy farms in Illinois. 1971. Pestic. monitor. J., 4, 233-237
- Naber, E.C. (1977). The impact of contamination by organo-chlorine insecticides on poultry nutrition and feeding. Fedn Proc., 36, 1880-1887
- Nichol, A.W., Elsbury, S. & Rousseaux, C.G. (1981). Porphyrin accumulation in sheep bones associated with 1,2,4 trichlorobenzene. Bull. environ. Cont. Toxicol., 27, 72-78
- Onley, J.H., Giuffrida, L., Watts, R.R., Ives, N.F. & Storherr, R.W. (1975). Residues in broiler chick tissues from low level feedings of seven chlorinated hydrocarbon insecticides. J. Ass. Off. anal. Chem., 58, 785-792
- Page, R.K., Fletcher, O.J., Vezey, S., Bush, P. & Booth, N. (1978). Effects of continuous toxaphene feeding to white leghorn layers. Avian Pathol., 7, 289-294
- Palermo, D., Lo Muzio, F. & Gentile, G. (1978). Residui di pesticidi clororganici nelle carni degli equini allevati a Foggia. Archo vet. ital., 29, 69-70
- Palmer, W.A., Dingle, J.H.P. & O'Neill, G.H. (1977). Residues of dioxathion in adipose tissue of cattle subjected to multiple dippings. Austr. J. exp. Agric. Anim. Husb., 17, 20-24
- Putnam, E.M., Brewer, R.N. & Cottier, G.J. (1974). Low level pesticide contamination of soil and feed and its effect on broiler tissue residues. Poult. Sci., 53, 1695-1698
- Reed, D.L., Booth, N.H., Bush, P.B., Goetsch, D.D. & Kiker, J. (1977). Residues in broiler chicken fed low levels of Hexachlorobenzene. Poult. Sci., 56, 908-911
- Richou-Bac, L., Restitut, A. & Pantaleon, J. (1975). Residues des pesticides dans les aliments pour animaux, evaluation des normes de tolerance. Bull. Acad. vet. Fr., 48, 483-498
- Richou-Bac, L. & Cumont, G. (1971). Les residues de pesticides dans les viandes, les graisses animales et les vegetales. Revue Med. Vet., 4, 391-413
- Rousseau, M., Pourtailler, J. & Taliencio, Y. (1972). Causes des pollution des oeufs et des viandes de la poule domestique (*Gallus-Gallus L.*) par des residues de pesticides organo-chlores. Bull. Acad. vet. Fr., 45, 39-45
- Shanks, V. (1975). Fenclorophos residues in the milk of cows and in the tissues of sheep after pour-on treatment. N.Z. J. exp. Agric., 3, 37-41

- Siegel, H.S. & Latimer, J.W. (1981). Effect of sex hormones on the retention of dieldrin in the fat of growing chickens. Poult. Sci., 60, 2210-2217
- Siegel, H.S., Latimer, J.W., Drury, L.N. & Loy, E.W. (1976). Accumulation and depletion of dieldrin in visceral fatty tissue of broilers. Poult. Sci., 55, 2319-2326
- Smart, N.A., Hill, A.R.C. & Roughan, P.A. (1974). Chlorinated hydrocarbon pesticides residues in foodstuffs: comparison of methods. J. Ass. off. anal. Chem., 37, 153-164
- Spencer, D.A. (1971). Movement of chemicals through the environment. J. Dairy Sci., 54, 706-712
- Stull, J.W., Brown, W.H. & Whiting, F.M. (1971). Correlation between pesticides levels in milk and carcasses of dairy cows. Bull. environ. Cont. Toxicol., 6, 568-569
- Swann, R.L., Sauderhoff, M.W., Laskowski, D.A. & Braun, W.H. (1977). The fate of phenyl N,N'-dimethylphosphorodiamidate in animals. In Fate of pesticides in large animals (ed. G.W. Ivie and H.W. Dorough), pp. 217-231
- Tonkelaar, E.M. den, Verschuuren, H.G., Bankovska, J., Vries, T. de, Kroes, R. & Esch, G.J. van (1978). Hexachlorobenzene toxicity in pigs. Toxic. appl. pharmac., 43, 137-145
- Tseng, Y-C.L. & Menzer, R.E. (1974). Effect of hepatic enzymes inducers (phenobarbital and dieldrin) on the in vivo and in vitro metabolism of dicrotophos, dimethoate and phosphamidon in mice. Pestic. Biochem. Physiol., 4, 425-437
- Vzoukwu, M. & Sleight, S.D. (1972). Effects of dieldrin in pregnant sows. JAVMA, 160, 1641-1643
- Varela-Alvarez, H. (1973). Certain physiological factors affecting organochlorine pesticides metabolism in ovines. Diss. Abstr. Int., 33B, 2871-2872
- Viviani, R. (1973). Aspetti biochemici nell'ecologia dei pesticidi. Atti Soc. ital. Sci. Vet., 27, 129-135
- Vos, R.H. de, Bouwman, J. & Engel, A.B. (1972). Residues of organochlorine pesticides in broilers from feed fortified with known levels of these compounds. Neth. Pestic. Sci., 3, 421-432
- Vreman, K., Tuinstra, L.G., Hoek, J. van den, Bakker, J., Roos, A.H., Visser, H. de & Westerhuis, J.H. (1976). Aldrin, Heptachlor and β HCH to dairy cows at 3 oral dosages. 1-residues in milk and body fat of cows early and late in lactation. Neth. J. agric. Sci., 24, 197-207

- Wagstaff, D.J., McDowell, J.R. & Paulin, H.J. (1980). Heptachlor residue accumulation and depletion in broiler chickens. Am. J. vet. Res., 41, 765-768
- Waldron, A.C. & Naber, E.C. (1974). Importance of feed as an unavoidable source of pesticide contamination in poultry meat and eggs. 1. Residues in feedstuffs. 2. Residues in eggs and tissues. Poult. Sci., 53, 1359-1371 and 1428-1435
- Walker, A.I.T., Stevenson, D.E., Robinson, J., Thorpe, E. & Roberts, M. (1969). The toxicity and pharmacodynamics of dieldrin (HEOD): two years oral exposure of rats and dogs. Toxic. appl. Pharmac., 15, 345-373
- Ware, G.W. (1978). The pesticide book. W.H. Freeman and Co., S. Francisco
- Ware, G.W., Estes, B., Buck, N.A. & Marchello, B.A. (1978). Decline of DDT residues in beef fat after 8 years of DDT moratorium in Arizona. Bull. environ. Cont. Toxicol., 20, 28-30
- Whiting, F.M., Brown, W.H. & Stull, J.W. (1972). Pesticide residues in tissues of first-born calves from dams on long time, low DDT intake. J. Dairy Sci., 55, 1499-1501
- Whiting, F.M., Brown, W.H. & Stull, J.W. (1973). Pesticide residues in milk and tissues following long, low 2,2 bis (p-chlorophenyl)-1,1,1 trichloroethane intake. J. Dairy Sci., 56, 1324-1328
- Whiting, F.M., Stull, J.W. & Brown, W.H. (1972). Pesticide depletion in dairy cows following long term exposure. Bull. environ. Cont. Toxicol., 7, 75-79
- WHO (1972). Evaluation of some pesticide residues in food. WHO Pesticide Residues Series No. 1
- WHO (1973). Evaluation of some pesticide residues in food. WHO Pesticide Residues Series, No. 2
- WHO (1974). Evaluation of some pesticide residues in food. WHO Pesticide Residues Series, No. 3
- WHO (1975). Evaluation of some pesticide residues in food. WHO Pesticide Residues Series, No. 4
- WHO (1976). Evaluation of some pesticide residues in food. WHO Pesticide Residues Series, No. 5
- WHO (1971). Pesticide residues in foods. WHO Tech. Rep. Ser., No. 474

- WHO (1972). Pesticide residues in foods. WHO Tech. Rep. Ser., No. 502
- WHO (1973). Pesticide residues in foods. WHO Tech. Rep.Ser., No. 525
- WHO (1974). Pesticide residues in foods. WHO Tech. Rep. Ser., No. 545
- WHO (1975). Pesticide residues in foods. WHO Tech. Rep. Ser., No. 574
- WHO (1976). Pesticide residues in foods. WHO Tech. Rep. Ser., No. 592
- WHO (1977). Pesticide residues in foods. WHO Tech. Rep.Ser., No. 612
- WHO (1980). Peripheral neuropathies. WHO Tech. Rep. Ser., No. 654
- WHO (1980). Resistance of vectors of disease to pesticides. WHO Tech. Rep.Ser., No. 655
- Woodham, D.W., Bond, C.A., Ahrens, E.H. & Medley, J.G. (1975). The cumulation and disappearance of mirex residues. III. In eggs and tissues of hens fed two concentrations of the insecticide in their diet. Bull. environ. Contam. Toxic., 14, 98-104

