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PREPARATORY STUDY FOR ESTABLISHING CRITERIA (EXPOSURE / EFFECT RELATIONSHIPS) FOR HUMANS ON ORGANOCHLORINE COMPOUNDS, i.e. PESTICIDES

2nd Series

Rapporteur: M. MERCIER
(Université Catholique de Louvain, Belgium)

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INTRODUCTION

The scope of the study is intended to encompass the present state of knowledge concerning the nature, extent and consequences of human exposure to organochlorine compounds, i.e. pesticides, 2nd serie.
It has been decided to take the following compounds into consideration :

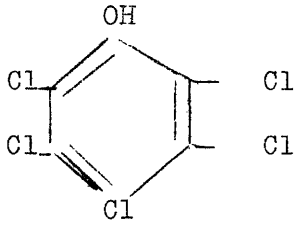
- Pentachlorophenol
- Pentachloronitrobenzene
- 2,4 D and 2,4,5 T
- MCPA, MCPB, Mecoprop and dichlorprop
- Methoxychlor
- Hexachlorobenzene
- Endosulfan

The state of art will be made of the situation regarding the effects of these compounds contributing to health hazards to humans.

Therefore, account will be taken of the following aspects which will be discussed.

1. Absorption, distribution and excretion
2. Metabolic pathways
3. Toxic effects : acute and more especially chronic effects
4. Biochemical effects
5. Carcinogenic effects
6. Mutagenic effects
7. Embryotoxic effects
8. Gaps existing in the information will be put forwards and advice will be given for stimulating and orienting further research.

PENTACHLOROPHENOL



C_6HCl_5O (266.5)

Pentachlorophenol is also known as PCP, pentapenchlorol.

Trade marks include "Dowicide 7" "Dowicide G " (Dow chemical Company); "Santophen 20" "Santobrite" (The Monesanto Company).

It was introduced in 1936 for timber preservation. It is an insecticide used for termite control and a fungicide used for the protection of timber from fungal rots and wood-boring insects. It is strongly phytotoxic and is used as a pre-harvest defoliant and as a general herbicide.

It is used as such or formulated in oil solution. "Santobrite" and "Dowicide G" are the technical sodium pentachlorophenate.

I PHYSICO CHEMICAL PROPERTIES

Colourless crystals with a phenolic odour of m.p. 191°C v.p. 0.12 mm Hg at 100°C; it is volatile in steam. Its solubility in water is 20 ppm at 30°C and it is soluble in most organic solvents though of limited solubility in CCl_4 and in paraffinic petroleum oils. The technical product is a dark grayish powder or flakes of m.p. 187 to 189°C

The sodium salt forms buff flakes with one mole of water of cristallization, its solubility in water at 25°C is 33 g/100 g and it is insoluble in petroleum oils. Its aqueous solution has an alkaline reaction.

PCP is non-inflammable. Through non-corrosive in the absence of mixture, its oil solution cause a deterioration of rubber but synthetic rubbers may be used in equipment and protective clothing.

II BIOLOGICAL DATA ON LABORATORY ANIMALS

1. METABOLISM

From experiments in the rabbit and the rat (Deichmann, 1942; Betts, 1955) it had been proposed that PCP was excreted unchanged and unconjugated from the body.

In the mouse, however, PCP has been found to be excreted both free and conjugated, unconjugated tetrachlorbydroquinone has been identified as one excretion product in this species (Jacobson, 1971).

In a recent study (Ahlborg, 1974), excretion of (^{14}C) pentachlorophenol in the urine of rats and mice after oral and intraperitorical administration (10 to 25 mg/kg) was studied.

Intraperitorical injection of ^{14}C PCP was followed by a rapid excretion of the major part of the radioactivity in urine, about 70% being recovered after 24 hr. Urinary excretion was lower after oral administration, as had also been noted in the rat by Larsen, 1972.

Tetrachlorohydroquinone has been demonstrated to represent 24% of the 24 hr of activity in the mouse, but only 5% in the rat. Tetrachlorohydroquinone has also been found in the urine of workers occupationnally exposed to PCP.

The pharmacokimetic profile of PCP has recently been redetermined in rats and monkeys (Braun, 1976).

Rats given 10 mg/kg (^{14}C) PCP orally eliminated more than 90% of the radioactivity with a half-life of 17 and 13 hr for males and females respectively.

After the same dose to monkeys, the elimination half-lives of the radioactivity were 72 and 84 hr for males and females, respectively. Both species eliminated the radioactivity primarily via the urine. The radioactive substances in the urine were identified as PCP, tetrachlorohydroquinone, and PCP glucuronide in rats, but as only unchanged PCP in monkeys.

Throughout the study in both rats and monkeys, the liver and kidneys contained the highest concentration of radioactivity. Plasma concentrations of radioactivity were high relative to other tissues, apparently due to reversible binding to plasma proteins.

From these studies, it can be concluded that PCP is rapidly excreted mostly in the urines, and does not accumulate in tissues or fat.

PCP is metabolized, mainly by conjugation with glucuronic acid and by replacement of the chlorine atom in para position by a phenolic group.

2. ACUTE TOXICITY

a) Laboratory animals

Pentachlorophenol or its sodium salt, when absorbed in sufficient quantity, produced in all species of animals studied (dogs, rabbits, rats, guinea pigs), an acute toxic state characterized by increased blood pressure, hyperpyrexia, hyperglycemia and glycosuria, hyperperistalsis, an increased and later a diminished urinary output, and rapidly developing motor weakness. In addition to these signs and symptoms, dying animals showed complete collapse and asphyxial convulsive movements. Rigor mortis was immediate and profound. The post-mortem evidences of injury were not specific and consisted largely of extensive damage to the vascular system, with heart failure and involvement of the parenchymatous organs. Pentachlorophenol applied cutaneously caused a more or less pronounced edema of the skin, which in about a week became dry and wrinkled. Slight cracks developed and hair was lost completely from the treated areas, but the hair follicles and the deeper structures of the skin apparently suffered no permanent injury (Deichmann, 1942).

The following tables summarize the results obtained after a single administration by different routes or several animal species.

The toxicity of pentachlorophenol and sodium pentachlorophenate for rabbits (Decha, 1939)

Compound	Concentration and solvent	Lethal doses in terms of pentachloro-phenol	Time till death	Comments
		Cutaneous administration		
Pentachlorophenol	5% in olive oil	mgm/kgm	hrs.	180 mgm/kgm produced no apparent ill effects
Pentachlorophenol	5% in 95% ethyl alcohol			150 mgm/kgm produced no apparent ill effects
Pentachlorophenol	10% in 95% ethyl alcohol			111 mgm/kgm produced acute illness and local damage
Pentachlorophenol	11% in olive oil			450 mg/kgm produced acute illness
Pentachlorophenol	10% in corn oil			326 mgm/kgm produced no apparent ill effects
Pentachlorophenol	5% in Stanolex fuel oil N°1	60-70	1½-4	
Pentachlorophenol	5% in Shell dione oil	110-120	5-6½	
Pentachlorophenol	5% in Std. oil (Ind.) pale paraffin oil			150 mgm/kgm produced acute illness and local damage
Pentachlorophenol	5% in Stanolex furnace oil	90-100	1½-3	
Pentachlorophenol	5% in Shell N°3 fuel oil	130-170	6	
Pentachlorophenol	1.8% in pine oil	40-50	9-22	
Na pentachlorophenate	10% aqueous	250	3-8	

The toxicity of pentachlorophenol and sodium pentachlorophenate for rabbits (cont'd)

Compound	Concentration and solvent	Lethal doses in terms of pentachlorophenol	Time till death	Comments
		Oral administration		
Pentachlorophenol	5% in Stanolex fuel oil	70–90	2–5	
Pentachlorophenol	11% in olive oil	100–130	10–16	
Na pentachlorophenate	5% aqueous	250–300	3–6	
		Subcutanous administration		
Pentachlorophenol	5% in olive oil	75–85	3–6	
Na pentachlorophenate	10% aqueous	100	7	
		Intravenous administration		
Na pentachlorophenate	2% in water	22–23	1½–4	

The general and local effects produced by the application of solutions of pentachlorophenol to the skin of animals vary with the solvent employed as the vehicle. With due regard to duration of exposure and the area of skin involved, the minimal lethal dose of the compound for rabbits when dissolved in various materials and applied cutaneously in a single dose was as follows: 39 mg per kg in pine oil; 50 mg per kg in STanolex Fuel Oil N° 1; 90 mg per kg in Stanolex Furnace Oil; 110 mg per kg in Shell Dione Oil; and 350 mg per kg in olive oil.

The smallest dose of pentachlorophenol in olive oil which resulted in the death of a rabbit when administered orally was 110mg per kg; when injected subcutaneously, 50 mg per kg.

The smallest lethal dose of sodium pentachlorophenate in aqueous solution or in 1% NaCl solution, when applied to the skin was 257 mg per kg expressed in terms of free pentachlorophenol; when administered by mouth the corresponding dose was 218 mg per kg, and when given intravenously, 22 mg per kg.

The toxicity of pentachlorophenol and sodium pentachlorophenate for rats

Compound	Concentration and solvent	Number of rats used	LD_{50}	Time till death
	Oral administration			
			mg/kg	hrs
Pentachlorophenol	0.5% in Stanolex fuel oil	80	27.3	3-19
Pentachlorophenol	1% in olive oil	60	77.9	3-11
Na pentachlorophenate	2% in water	60	210.6	2-13
	Subcutaneous administration			
Na pentachlorophenate	2% in water	80	66.3	2-8

Pentachlorophenol is repellant to animals; the daily food intake of rats decreased when the diet contained pentachlorophenol, and cats refused to eat salmon similarly treated (Deichmann, 1942); Cattle avoided pasture treated with pentachlorophenol (Grigsby, 1950); however, it has been observed that cattle will drink almost anything, including pentachlorophenol solutions, when they are thirsty (Spencer, 1957).

Spencer, 1957 reported the death of a Hereford cow which had imbiled a five-percent solution of pentachlorophenol in kerosene. At necropsy, eight hours after death, a mild reddening of the mucosa of the rumen, abomasum, and small intestines was observed; the surface of the liver and the cortex of the kidney showed numerous pale areas; all tissues had an oily odor. Diagnosis was poisoning by consumption of pentachlorophenol in kerosene, but it was noted that kerosene alone would have been toxic to the animal. It was also noted that pentachlorophenol was a tissue fixative and would prevent fermentation in the rumen.

Walters, 1952 drenched swine (30 g of pentachlorophenol) and sheep (23 g of pentachlorophenol) with pentachlorophenol solutions. The kidney, liver, and spleen of the swine showed some cell damage, but not severe enough to cause death. No harmful effects were noted in the kidney or liver of the ewes or the kidneys of the lambs; some detrimental effect was noted in the liver of the lambs..Schipper, 1961 confined swine to farrowing pens that had been treated with a four-percent pentachlorophenol petroleum distillate formulation. Pig mortalities were high and increased with the length of the confinement period. Lesions were observed in the kidney, liver, spleen, stomach and the intestinal and respiratory tracts. Two sows confined to a farrowing crate treated three days earlier with three applications of undiluted pentachlorophenol showed signs of irritation within five hours after confinement and died within 24 hours; extensive abdominal burns and necrosis were evident. The study showed that pentachlorophenol was extremely toxic to young swine, and that the degree of toxicity was proportional to the age of the pig. Direct contact with the freshly pentachlorophenol-treated lumber was responsible for toxicosis, and a liberal amount of bedding would have prevented the problem. Blevins, 1965 recorded an incident of ten pigs dying one day after birth. The gilt had been housed in an area treated two days earlier with a solution of pentachlorophenol in crankcase oil; the owner had exceeded the application dosage recommended by the manufacturer.

Lollar, 1944 observed no local or systemic toxicity effects with dogs and horses that had worn leather collars or harness containing 0.25 to 0.50 percent pentachlorophenol.

3. CHRONIC TOXICITY

A) Local and systemic effects resulting from repeated cutaneous application of pentachlorophenol and sodium pentachlorophenate (Deichmann, 1942)

- A one per cent solution of pentachlorophenol in mineral oil was applied to the skin of two rabbits in doses of 10 cc., corresponding to about 40 mg per kilogram. At the end of four hours the excess material was wiped off with cotton and, without further washing, the animals were returned to their cages. Both animals survived 21 successive daily treatments without illness, loss of weight or injury to the skin.

- Doses ranging from 10 to 50 mg of pentachlorophenol per kilogram, as a 4% solution in Stanolex Fuel Oil, were applied to the skin of the back of rabbits once or twice a week, for periods ranging from 6 to 61 weeks. The compound was not washed off. The local effects produced were the same as those induced by a single large dose. A rise in rectal temperature of 1-2°C (measured 8 hours after treatment) occurred occasionally after a dose of 10 mgm. per kilogram, and regurlarly after larger doses. Erythrocyte counts, differential counts, and hemoglobin determinations were made monthly . The fluctuations observed did not exceed those which occurred in untreated animals. There were no significant changes in body weight. Notwithstanding the absence of specific signs of poisoning, 8 of the 20 rabbits died during the experiment. Several rabbits were killed for pathological examination; the gross and hispathological tissue changes were in direct relation to the size of the dose and the period of treatment.

- Similar experiments were made with the sodium salt (2% solution). On 32 successive days a rabbit was given the dose of 63 mg per kilogram. At no time did this animal show illness or injury to the skin. Another rabbit received two doses (on 2 successive days) of 113 mg per kilogram This animal died four hours after the second treatment. A third rabbit died from acute intoxication after the thirteenth application of 111 mg per kilogram, applied over a period of 43 days.

- Ten cc. doses of a 1% solution of the sodium salt (about 40 mg/kg) were applied daily (except Sundays) for 100 consecutive days to the skin of 6 rabbits. In the case of 3 rabbits the solution was allowed to remain on the skin for 30 minutes, and in 3 others for one hour, after which the

compound was washed off with soap and water. The treated areas occasionally showed mild irritation but no wrinkling or cracking of the skin and no loss of hair. The gain in body weight was normal, and there were no fatalities.

- Blood analysis have demonstrated that repeated application of 100 mg pentachlorophenate results on demonstrable absorption, the blood level rising to about 0.45 mg %

B) cumulative effects from oral doses

a. Feeding experiments were performed on rats, cats and rabbits (Deichmann 1942).

a.1. PCP, dissolved in 95% ethanol, was fed to two groups of 10 rats each. The first group was fed over a period of 26 weeks, each rat ingesting daily approximately 5 mg of the compound.
These animals neither gained nor lost weight during this period; their failure to grow apparently resulted from a reduction in their intake of food.
The second group was fed over a period of 28 weeks, each rat ingesting about 3.9 mg of PCP daily. These animals did not grow at the normal rate; post-mortem examination of their tissues revealed no gross and only insignificant histological abnormalities.

a.2. Cats
4 cats received PCP or its sodium salt in doses equivalent to 1.25 and 2.5 mg/kg for about 10 weeks.
The animals showed some loss of appetite and loss of weight, but none of the cats displayed signs of PCP poisoning. At the end of the experiment, their blood contained about 0.3 to 1.8 pm of PCP per 100 cc.

a.3. Rabbits
Five rabbits were given a daily oral dose of 35 mg pentachlorophenol per kilogram (about 1/8 of the lethal dose) as a 0.5% solution of the sodium salt, for 15 days. During the following 19 days a 5% solution was used and the dose was raised gradually to 600 mg per kilogram (about twice the lethal dose). Rectal temperatures, blood counts and determinations of hemoglobin and of blood sugar were made every second or third day, six hours after the treatment. The animals were weighed weekly.

One animal died after having ingested a total of 1.9 grams of pentachlorophenol per kilogram; two died after the ingestion of 2.9 grams per kilogram, and two others after 3.9 grams per kilogram. This severe treatment caused loss of body weight, a very slight reduction in the number of erythrocytes and a parallel drop in the concentration of hemoglobin. Leukoxytes and rectal temperatures fluctuated but did not exceed the limits of variations observed in control rabbits.
The most definite evidence of the establishment of tolerance to the compound is the finding of blood concentrations of 14, 21, 22, 36 and 39 mgm% of pentachlorophenol in animals that died. After single lethal doses of pentachlorophenol, the concentration of pentachlorophenol in the blood of rabbits did not exceed 8.5 mgm%.

b. In another serie of experiments, chronic effects following administration of small doses for prolonged periods of time have been examined on rabbits by different routes of administration (Mc. Gavack, 1941).

Sodium pentachlorophenate was subcutaneously administered daily to 3 groups of 6 rabbits each in 1/20, 1/10 and $\frac{1}{4}$ the minimum lethal dose (M. L. D. 275 mg/kg) respectively and intraperitonecally daily in 1/10 the M.L.D. (150 mg/kg) to a single group of 3 animals.

The drug was continued until lethal except in the group receiving 1/20 M.L.D., the members of which were sacrified at the end of 60 days.

Of these animals, three lost weight and showed other evidences of poisoning, while the remaining three actually gained weight and gave no clinical evidence during life of functional or organic disease. The animals receiveing 1/10 M.L.D. subcutaneously died after an average total dosage of 401.5 mg of sodium pentachlorophenate. Those receiving $\frac{1}{4}$ M.L.D. succumbed after an average of 7.8 injections and a total dosage of 546.0 mg per kg of body weight.

The drug was less well tolerated by the intraperitoneal route, as 15.0 mg per kg daily proved fatal in each of 3 animals after an average of 11 days' administration and an average total dose of 165 mg/kg.

The clinical picture was similar to that seen in the acute intoxication and death always occurred in convulsive seizures, although these were not as vigourous in the chronically affected as in the acutely poisoned rabbit.

When 1/10 M.L.D. was given, a secondary anemia of moderate degree was the rule.

Leucopenia with relative lymphocytosis usually accompanied the drop in erythrocytes.

In 2 of the 6 rabbits receiving daily injections of 1/20 M.L.D., a polycythemia developed.
The blood chemical analysis were within normal values.

Post-mortem findings may be summarized as follows:
The right heart was always moderatly to markedly dilated; the left usually contracted;
Histologically, slight focal lymphocytic infiltration was occasionally noted;
Isolated patechial hemorrhages were present in the thymus in all but one case.
Partial collapse of the lungs, multiple parenchymal hemorrhages and diffuse congestion were present in most cases. Focal pneumonic was a common finding, and moderate hypertrophy of the pulmonary artery is recorded in 90% of the animals;
Cloudy swelling of the liver in varying degree was present in every case. In some rabbits, vacuolization and fatty infiltration were noted;
The kidneys showed a picture of cortical congestion and hemorrhage on about one-third the cases.

4. CARCINOGENIC EFFECTS

Results of the long-term studies previouslydescribed did not reveal any increase in the tumor incidence of PCP treated animals.

From a study conducted by the National Cancer Institute, PCP has been considered as non tumorogenic in the one animal species studied.

5. MUTAGENIC EFFECTS

No information was found in the litterature.

6. EMBRYOTOXIC EFFECTS

The effects of purified and commercial grade PCP on rat embryonal and fetal development have been evaluated (Schetz, 1974).

The study was designed to determine whether PCP influences the development of the embryo and the foetus, and whether the non-phenolic in a commercial preparation of PCP may influence the development of the embryo and foetus.

Description of Test Materials

	Pentachlorophenol	
	Commercial grade	Purified
Phenolies (%)		
Pentachlorophenol	88.4	98+
Tetrachlorophenol	4.4	0.27
Trichlorophenol	0.1	0.05
Higher cholinated phenoxyphenols	6.2	0.5
Nonphenolies (ppm)		
Dibenzo-p-dioxins		
2,3,7,8-tetrachlorodibenzo-p-dioxin	0.05	0.05
Hexachlorodibenzo-p-dioxin	4	0.5
Heptachlorodibenzo-p-dioxin	125	0.5
Octachlorodibenzo-p-dioxin	2500	1.0
Dibenzofurans		
Hexachlorodibenzofuran	30	0.5
Heptachlorodibenzofuran	80	0.5
Octachlorodibenzofuran	80	0.5

Doses of 0, 5, 15, 30 and 50 mg/kg/day of commercial grade or purified PCP were administered by gavage to groups of 20 - 40 bred rats on days 6 - 15 inclusive of gestation. It must be noted that 50 mg/kg represents the maximum tolerated dose.

Additional groups of rats were given 0 or 30 mg PCP/kg/day on days 8 - 11 or 12 - 15 of gestation. All rats were observed daily throughout pregnancy and were weighed on days 6, 15 and 21 of gestation.

Following the administration of PCP, signs of embryotoxicity and fetotoxicity, such as resorptions, subcutaneous edema, dilated ureters and anomalies of the skull, ribs, vertebrae and sternebrae were observed at an incidence which increased with increasing the dose. Purified PCP was slightly more toxic than the commercial grade. The developing rat embryo

is most susceptible to the toxic effects of a given dose of PCP during the period of early organogenesis.

The no-effect dose level was 5 mg of the commercial grade of PCP/kg/day.

A study conducted on hamsters fed 1.25 - 20 mg PCP/kg/day led to similar conclusions (Hinkle, 1973).

In a very recent experiment, (Courtney, 1976), 75 mg/kg PCP were administered to the CD rat on gestational days 7 - 18. The only fetal effect was a slight but significant decrease in fetal body weight.

7. SPECIAL EFFECTS

7.1 Effects of PCP on hepatic drug metabolism and porphyria related to contamination with chlorinated dibenzo-p-dioxins (Goldstein, 1973)

The effects of technical pentachlorophenol (PCP) containing a relatively high concentration of hexa-, hepta-, and octachlorodibenzo-p-dioxins, polychlorinated ethers, and polychlorinated dibenzofurans were compared with those of relatively pure (99%) PCP on hepatic drug metabolizing enzymes and the development of hepatic porphyria in the rat. The products, are among the most toxic compounds known. Female weanling rats were fed 0, 20, 100, and 500 ppm pure or technical PCP and sacrified at 8 months. Technical PCP increased urinary prophyrin excretion in 33% of the rats fed 100 or 500 ppm after 6 to 8 months of treatment. At sacrifice, the livers of these rats contained large amounts of uroporphyrins. None of the rats fed pure PCP became porphyric. Technical PCP induced aryl hydrocarbon hydroxylase (15, 35, and 43 - fold at 20, 100, and 500 ppm), glycuronyl transferase (4-, 5-, and 6- fold at 20, 100 and 500 ppm), cytochrome P-450 (2- and 3-fold at 010 and 500 ppm), microsomal heme (80 and 120% at 100 and 500 ppm), and liver weight (35% by 500 ppm).

Pure PCP had little effect on the liver, producing a 3-fold increase in glucuronyl transferase at 500 ppm, slight but insignificant increases in aryl hydrocarbon hydroxylase (2-fold), cytochrome P-450 (40%) and microsomal heme (40%), and no effect on liver weight. Neither pure nor technical PCP affected N-demethylase or δ-aminolevulinic acid synthetase. Even at the 20 ppm dose, technical PCP produced greater liver effects than 500 ppm pure PCP. In contrast, both technical and pure PCP reduced body weight gain comparably at 500 ppm without depressing food consumption. The prophyria

and other major liver changes induced by technical PCP are apparently due to contaminants rather than PCP, probably the chlorinated dibenzo-p-dioxins, some of which are known to induce porphyria, cytochrome P-448, glycuronyl transferase, and aryl hydrocarbon hydroxylase but not N-demethylase.

7.2 Effects of PCP on oxidative phosphorylation

Numerous experiments have shown that PCP is a powerful uncoupler of oxidative phosphorylation (Weinbach, 1957, 1964, 1966, 1966b, 1968; Bakker, 1974; Nichols, 1967), but does not affect substrate-linked phosphorylation (Buffe, 1963). In contrast to 2.4-dinitrophenol, it strongly inhibits both mitochondrial and myosin adenosine triphosphatase.

It has also been shown that PCP inhibits creatine phosphate synthesis as well as stimulating oxygen consumption in brain slices from rat (Cremer, 1961).

In vivo studies with fish have pointed out that PCP inhibits the enzyme aldolase, lactate dehydrogenese, glutamate exalacetate transaminase and glutamate pyruvate transaminase, yet activates isocitrate dehydrogenase (Kruger, 1966).

III BIOLOGICAL DATA ON HUMANS

1. ACUTE EFFECTS

Hayes, 1963 and Thienes, 1964 have described the effects of pentachlorophenol on man with the inclusion of data on fatal cases of accidental and occupational poisoning. The compound is absorbed by the skin and by inhalation. Irritation of the eyes, nose and throat can occur. The threshold limit value of pentachlorophenol in air is 0.5 mg/m^3, as documented by the American Governmental Industrial Hygienisis. The exact dosage required to produce illness in man is not known. Symptoms occur at concentrations of four to eight mg (40 to 80 ppm) per 100 ml of blood. Pentachlorophenol is excreted unchanged in the urine with three to ten ppm occurring in non-fatal cases. Fatal cases are preceded by high temperatures, sweating, dehydration, rapid pulse, and early coma. At death, 20 to 140 ppm of pentachlorophenol may be present in the tissue; 28 to 96 ppm have been observed in post-mortem urine. Concentrations of 97 ppm in blood from the lung; 62 ppm in the

liver; 46 ppm in blood from the liver; and 84 ppm in the kidney.

Gordon, 1965 reported nine cases of pentachlorophenol human poisonings for the years 1953 to 1956; five died within 16 to 30 hours after onset of symptoms. Three of the five fatal cases occurred after applying sodium pentachlorophenate in the sugarcane or pineapple fields, one was caused by treating weeds around a factory area, and one occurred after preparing a pentachlorophenol formulation. The four cases which recovered over a period of one week to four months had either been spraying in the sugarcane or pineapple fields or working in a sawmill with lumber that had been treated with sodium pentachlorophenate. The clinical picture was similar in all cases except one. The onset was marked by abdominal pain, nausea, excessive sweating, and vomiting; temperature was moderately elevated, pulse rate was rapid, and respiration was increased. The exceptional case (completely recovered after four months in the hospital) was involved in the pentachlorophenol treatment of lumber; the work was constant and exposure had occurred over a long period of time with a gradual onset of illness. Two of the autopsies indicated gross congestion in the lungs and widespread intra-alveolar hemorrhage. The other autopsies were not fully documented. In all of the cases, improper dress, careless handling of the sprays, and laxity of proper supervision may have contributed to the problem.

Four families became ill after using water from a well for drinking and bathing pruposes (Uede, 1962); symptoms included fever, irritated throats, and red faces. Investigation revealed that four days prior to the illnesses, pentachlorophenol had been applied to a rice field near the well (distance about two meters). Analysis of the well water for pentachlorophenol content was made five days after the illnesses were noted and the pentachlorophenol content of the water was found to be 12.5 ppm. The affected families improved within a two - to three-day period after the well was declared "off limits".

An individual accidentally used an organic solvent containing pentachlorophenol to clean a paint brush, using unprotected hands during the cleaning process (Bevenue, 1967). The analysis of urine samples from this case showed an initial (sample obtained 48 hours after exposure) concentration of 236 parts per billion (PPB) of pentachlorophenol, which decreased gradually over a period of 30 days to a level of about 10% of the initial concentration.

An epidemic with two deaths occurred in a nursery in St. Louis after the use of PCP as a mildew preventive with the laundry detergent (Barthel, 1969).

In 1967, 20 newborn infants in a small hospital developed an unusual illness characterized by profuse, generalized diaphoresis, fever, tachycardia, tachypnea , heptomegaly and acidosis. Nine of these children were severely ill and 2 died (Robson, 1969); Amstrong, 1969). The autopsy revealed fatty infiltration of the liver and fatty degeneration of renal tubular cells. The intoxications have been produced by the percutaneous absorption of PCP which had been used in the laundering of the diapers and the infants' bed linen.

Two additional cases of fatal poisoning by PCP have been described involving individuals working at packaging PCP and consequently inhaling considerable amounts of dust particles of the chemical (Mason, 1965).

2. CHRONIC EFFECTS

Medical examination of workers engaged for periods of times ranging from 2 to 6 years in the production of sodium pentachlorophenolate has shown that some individuals suffered from a mild chronic chlorinated phenol poisoning. Signs of poisoning include acneiform occupational dermatitis of the face, functional nervous system and liver disorders, and changes in the blood (isolated heinz bodies in the erythrocytes, relative lymphocytosis, aneosinopenia, ...) (Vinogradova, 1973).

IV CONCLUSIONS

PCP is readily absorbed through the skin, lungs and gastrointestinal tract. It is rapidly excreted in urine, as unchanged pentachlorophenol together with its glucuronide and tetrachlorohydroquinone. It does not accumulate in tissues or fat. PCP is a rather toxic compound with an oral LD_{50} on rats of 27 mg/kg. The percutaneous LD_{50} is 105 mg/kg.

Signs of poisoning include hyperpyrexia, hyperglycemia, glycosuria, asphyxial convulsions, cardiovascular failure, rigor mortis immediate after death.

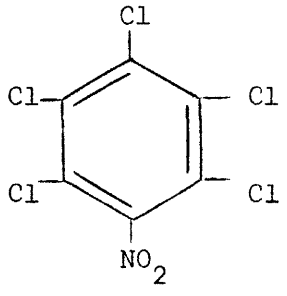
In vitro experiments have shown that the toxic effects are due to uncoupling of oxidative phosphorylation.

The data obtained on several animal species chronically treated with PCP are not sufficiently complete to allow the determination of a no-effect level.

Informations on the possible carcinogenic and mutagenic effects of PCP are rather poor.

When administered at high doses, PCP has been shown to be embryo- and fetotoxic, the no-effect level being 5 mg/Kg/day.

QUINTOZENE



$C_6Cl_5NO_2$ (295.5)

Quintozene is the common name approved by ISO, except Turkey (terrachlor) and USSR (P KhNB) and by BSI for pentachloronitrobenzene, it is also known as PCNB.

It was introduced, in the late 1930's as a fungicide by I.G. Farbenindustrie AG. Trade marks include "Brassicel" "Tritisan" (Farbwerke Hoechst AG), "Folosan", "Terrachlor" (Oline Mathison Chemical Corporation). Protected by DRP 682, 048.

Quintozene is a fungicide of specific use for seed and soil treatment effective against bunt of wheat, botrytis, Rhizoctonia and sclerotinia spp.

Storage of PCNB in the fat of rats has been evaluated on samples of subcutaneous and perirenal fat taken at termination of a three-month feeding study on rats (Finnegan, 1958).

It appeared that the ratio of fat storage of PCNB to the concentration fed is fairly linear and that the ratio is much lower than has been observed for other fat-soluble agricultural chemicals, like DDT. The PCNB concentrations in fat ranged from an average of 43 ppm for the 63.5 ppm diet to 1234 ppm for the 2500 ppm diet.

I PHYSICO CHEMICAL PROPERTIES

Colourless needles of m.p. 146°C, v.p. 133 x 10^{-4} mm Hg at 25°C, d^{25} 1.718. It is practically insoluble in water, soluble to about 2% in ethanol at 25°C.

Soluble in benzene, carbon disulphide, chloroform. It is of high stability in soil and compatible with all pesticides at pH7 or less. It is non-corrosive and stable in sunlight.

The technical product is 98.5% pure and of m.p. 142 to 145°C.

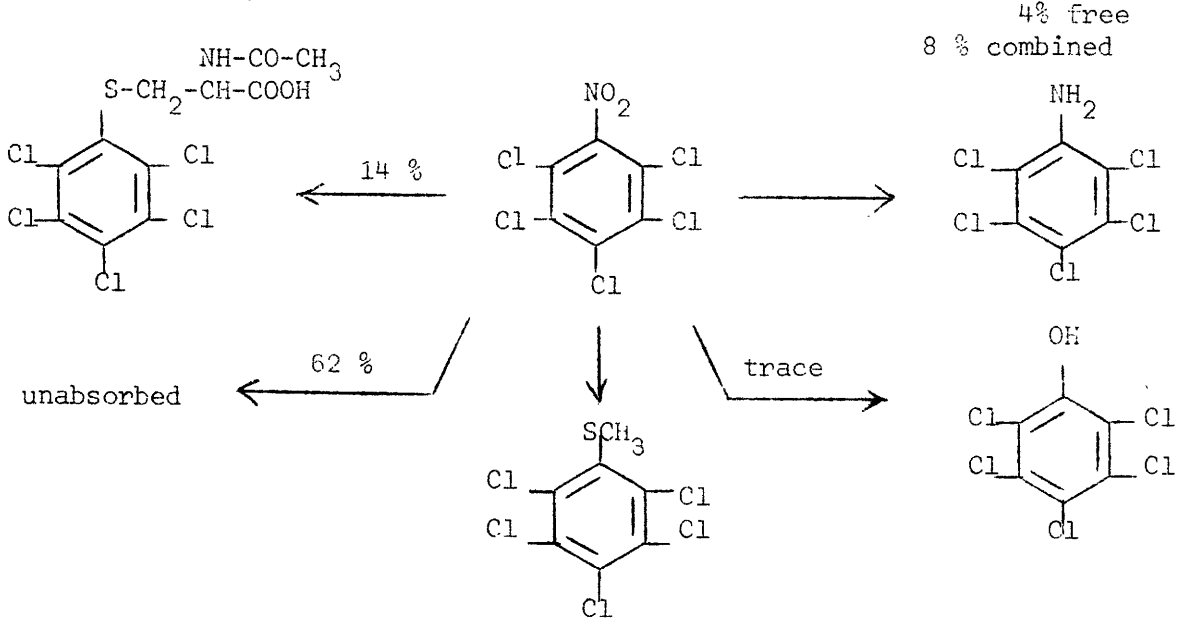
Some preparation contain hexachlorobenzene as an impurity (Borzelleca, 1971; Gonrsand, 1972). It is formulated as dusts, wettable powders, emulsifiable concentrates, pastes and formulations containing a mixture of pesticides. Some formulations contain 5-ethoxy-3-trichloromethyl-1,2,4,-thiadiazole in addition to the quintozene.

II BIOLOGICAL DATA ON LABORATORY ANIMALS

1. METABOLIC STUDIES

The metabolism of PCNB has been studied in rabbits (Bett , 1955).

An average of 62% of PCNB is unabsorbed and excreted in faeces. The metabolites recovered in the urine are respectively pentachloroaniline (12%) and N-acetyl-S-pentachlorophenylcysteine (14%). The formation of the mercapturic acid from PCNB involves replacement of the nitro group by an acetylcytryl group.



However, the method is based on the measurement of ^{38}Cl formed after irradiation of the sample with a neutron beam and certainly lacks specificity. St.John et al, in 1965, fed a cow with a diet containing 5 ppm PCNB for three days ; 45 % of the administered dose was eliminated as pentachloroaniline within four days of the last dose ; no PCNB, pentachloroaniline or methyl pentachlorophenol sulphide could be detected in the milk.

Dogs have been fed 5 and 1,080 ppm of technical quintozene in their diet for 2 years and their tissues analysed for residues (Kuchar, 1969). No quintozene has been found in the fat, muscle, kidney or liver tissues.

Pentachloroaniline has been found in fat and liver in quantities lower than 1 ppm for both dose levels. Methylpentachlorophenol sulphide has been found in all four tissues, at levels up to 2.5 ppm in fats of animals fed 1,080 ppm.

Another study has been conducted on rats fed 50 or 500 ppm technical quintozene for 7 months. Less than 1 ppm of either of the metabolites have been found in the fat of the rats fed 50 ppm and about 1 ppm of pentachloroalin and 5 ppm of methylpentachlorophenyl sulfide for the 500 ppm group.

The storage of PCNB in different tissues has been examined during a 3-generation reproduction study on rats, a two-year feeding study on beagle dogs and a 16 wk feeding study on cows (Borzelleca, 1971).

Storage of PCNB did not occur in tissues of either animal. The apparent storage of PCNB found in the fat or rats by Finnegan et al was, therefore due to the non-specific nature of the neutron activation method used.

However, trace amounts of pentachloroaniline and methylpentachlorophenol sulfide have been found in various tissues and fat.

During the course of a study on mice (Courtney, 1973), it has been shown that both metabolites are present in fetuses, indicating their transplacental passage.

From these metabolic studies, it can be concluded that PCNB, when absorbed from the gastrointestinal tract, is rapidly excreted and does not accumulate in tissues or fat.

PCNB is rapidly metabolized into pentachloroaniline and methylpentachlorophenol sulfide, traces of which can be found in tissues of treated animals as well as in foetuses of mother exposed to PCNB.

2. ACUTE TOXICITY

Animal	Route of Administration	Vehicle	Formulation	LD_{50} (mg/kg)	References
Rat	oral	oil	Technical (a) grade PCNB	1,710 ± 200 1,650 ± 170	Finnegan, 1958
Rat	oral	aqueous suspension	75% wettable powder	16.000	Finnegan, 1958
Rat	oral	aqueous suspension	technical grade PCNB	30.000	Wit, 1957
Rat	i.p.	aqueous suspention	technical grade PCNB	5.000	Wit, 1957
Rabbit	oral	oil	technical grade PCNB	800(b)	Finnegan, 1958
Dog	oral	oil	technical grade PCNB	2.500	Finnegan, 1958
Rabbit	percutaneous	Dimethylphtalate	technical grade PCNB	4.000(c)	Borzelleca, 1971

(a) Technical grade PCNB : Pentachloronitrobenzene 98.2%
Hexachlorobenzene 1.4%
Traces of tetrachlorobenzene and pentachlorobenene

(b) Erratic absorption from the gastroenteric tract in the rabbit

(c) No skin irritation.

3. CHRONIC TOXICITY

Short- and long-term studies on rats

- Young rats were fed diets containing 2.000 ppm of quintozene for 10 weeks. The males grew less than controls (Wit, 1957).

- Groups of 10 rats of each sex were fed a diet containing 0, 1.000, 5.000 and 10.000 ppm of quintozene for 3 months. A decreased growth rate was noted at 5.000 ppm and 10.000 ppm (Hoechst, 1964).

- In a three-month feeding study on rats, growth and survival were adversely affected in both sexes at a level of 5.000 ppm and in males growth was suppressed at 2.500 ppm.
Liver to body weight ratios were significantly elevated at all dietary levels except in the females at the lowest level (63.5 ppm).
No hematologic changes were seen and histopathologic changes were limited to fine vacuolization of liver cell cytoplasm at 5.000 ppm (Finnegan, 1958).

- In a similar two-year study, a tendency toward growth suppression occurred in females at levels as low as 100 ppm, although there was no definite gradient in growth depression with increasing dietary concentration. In male rates, growth depression occurred only at the 2.500 ppm level (Finnegan, 1958).

Long-term studies on dogs

- A one-year feeding study in dogs at 25, 200 and 1.000 ppm of quintozene in the diet showed no adverse effect on body weight or survival. The hematologic parameters were within normal values; the results of the histopathologic studies were negative except for the livers which showed cell enlargement with pale-staining cytoplasm.
However, there is no progressive increase in the severity of the lesion with increasing degree of dietary exposure to quintozene (Finnegan, 1958).

- Groups of four dogs of each sex were fed 0, 5, 30, 180 and 1.080 ppm quintozene in their diet for two years. There was no change in body weight gain or in food consumption.
Hematologic, as well as urine analysis revealed nothing remarkable.
Blood chemical determinations showed significantly higher SGOT values in females on 1.080 ppm at 3 months and higher SAP values in both sexes on

1.080 ppm at 12 months. The liver-to-body-weight values were higher at 1.080 ppm than for controls. Histologic examination of tissues showed cholestatic hepatosis with secondary bile nephrosis in minimal degree in dogs on 180 ppm and in moderate degree in dogs on 1.080 ppm.
No effect was noted in the dogs fed 5 and 30 ppm of quintozene (Borzelloca 1971).

- Groups of 3 dogs of each sex were placed on diets containing 0, 500, 1.000 or 5.000 ppm of quintozene for two years.
Liver damages occurred at all dose levels.
Severe liver damage with fibrosis, narrowing of hepatic cell cords, increased size of the periportal areas and thick leucocyte infiltration were seen at 5.000 ppm.
At the lower dose levels, the same changes were detected but at a lesser degree.
The 5.000 ppm level produced atrophy of bone- marrow and reduced haematopoiesis (Hoechst, 1968).

4. CARCINOGENIC EFFECTS

Studies in mice

a) oral administration

The tumorogenicity of quintozene was tested by continuous oral administration to both sexes of two hybrid strains of mice, started at the age of 7 days (Innes, 1969) Doses of 464 mg/kg were given by stomach tube from 7 days to 28 days of age. Thereafter, the animals were fed a diet containing 1206 ppm of quintozene, which was administered up to 78 weeks. (1206 ppm quintozene represents the maximal tolerated dose).
Results are summarized in the following table.

treatment	strain	Number of mice at term		Total mice necropsied		Weeks at term		Mice with hepatomas		Mice with pulmonary tumors		Mice with lymphomas		Total mice with tumors	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
Control	X(1)	73	83	79	87	-	-	8	0	5	3	5	4	22	8
	Y(2)	89	75	90	82	-	-	5	1	10	3	1	4	16	7
PCNB	X	14	18	18	18	78	78	2	4	2	1	2	0	5	5
	Y	16	17	17	17	78	78	10	1	1	0	1	1	11	2

(1) Strain X = (C 57 BL/6 x C3H/Anf)FI

(2) Strain Y = (C57 BL/6 x AKR) FI

There is a significantly elevated incidence of hepatomas in males of one strain and in females of another strain.

b) Skin application

10 males and 10 females mice were painted twice weekly for 12 weeks with 0.2 ml of a 0.3% solution of PCNB in acetone for 12 weeks. They were then painted with croton oil for 20 weeks and surviving mice were killed after a further 20 weeks without treatment. In the control group acetone containing no PCNB was painted.

Papillomata appaered in test animals after 5 to 8 weeks treatment with croton oil; the total number of tumors at the end of the croton oil treatment was 12 in 9 surviving controls and 50 in 13 surviving PCNB treated animals. Some of the skin tumors regressed after cessation of the treatment. One tumor in both control and treated groups progressed to a squamous-cell carcinoma (Searle, 1966).

5. MUTAGENIC EFFECTS

a) Dominant lethal test

Groups of 20 male mice were fed with 3 doses levels of PCNB for 7 weeks. Following treatment, each male was mated to two adult females weekly for 8 weeks . No consistent response occurred, suggesting that quintozene is non-mutagenic to the mouse by the dominant lethal procedure (Jorgensen, 1976).

b) Microbial assay systems

Pentachloronitrobenzene has been tested for its mutagenic potential in four microbial assay systems: the histidine reverse mutation system in five strains of salmonella typhimurium, an assay of mitotic recombination in a diploid strain of saccharomyces cerevisiae and relative toxicity assays in escherichia coli and bacillus.substilis. A mammalian metabolic activation system using the liver of rats which had been treated with Arochlor 1254 was used in all of the assays except the B. subtilis assay. The results were negative with PCNB.

6. EMBRYOTOXIC EFFECTS

- A 3 generation reproduction study has been conducted with CD-strain rats fed dietary levels of 0, 5, 50 and 500 ppm of quintozene.
The various estimated parameters: fertility, lactation, gestation and viability indices as well as weaning-weights were within normal values. No structural defect was noted in any pup. Histopathologic findings on F/3b offspring were entirely negative -(Borzelleca, 1971).

- Quintozene dissolved in corn oil, was administered orally to groups of 20 pregnant rates, from days 6 to 15 of gestation, at dose levels of 0, 8, 20, 50 and 125 mg/kg
No differences were observed between control and treated rates: number of corpora lutea, implantations, dead and resorbed fetuses, viable fetuses, fetal weight and sex, skeletal or soft tissue malformations (Jordan, 1973).

- Different samples of PCNB were evaluated for their teratogenic potential in the CD rat; all of there were administered as a corn-oil: acetone (9:1) solution from days 7 to 18 of the gestation.
The first sample (a) contained 11% hexachlorobenzene (HCB), the second one (b) contained 1% HCB and 0.1% pentachlorobenzene (PCB) and a third one containing 20 ppm HCB as the only detectable impurity.
Tetrachloronitrobenzene (TCNB) and pentachloraniline (PCA) were also investigated. The dose level used were respectively 500 mg/kg for sample a and c, 75 mg/kg for TCNB and 200 mg/kg for PCA.
No effect on maternal weight, liver weight, weight of fetuses, fetal viability and morphological development was seen (Courtney, 1976).

- Quintozene has been evaluated for its teratogenic potential in the C57 B1/6 and the CD-1 strain of mice (Courtney, 1976).
Pregnant C57 B1/6 mice were administered 500 mg/kg of PCNB, sample a (contaminated with 11% HCB), on days 7 - 11 of gestation.
Although there was a slight increase in fetal mortality, there was no significant difference in the number of live fetuses per litter.
However, the number of abnormal fetuses per litter was significantly increased: renal agenesis, anophtalmia and microphtalmia, cleft palates.
Pentachloroaniline (PCA) and TCNB, were administered on days 7 - 18 of gestation at dietary levels of 200 mg/kg.
Neither PCA nor TCNB significantly affected weight gain or maternal liver to body weight ratios, fetal body weights, fetal mortality or the number of abnormal fetuses. Since the compounds were tested at dose levels greater than could be anticipated from either the metabolism of PCNB or the amount obtained from treatment with contaminated PCNB, and since these compounds were tested for an extended period of fetal development, i.e., days 7 - 18 of gestation, they probably were not involved in the production of the malformations observed by treating the mice with contaminated PCNB as in sample a.

- Comparable studies were undertaken in the CD -1 mouse, treated on days 7 - 16 of the gestation. Contaminated PCNB (sample a) was administered at 250 and 500 mg/kg. There was a significant increase in the ratio of maternal liver to body weight. This value was not affected in mice treated with 250 mg/kg PCA.
Neither fetal weights nor mortality were affected by treatment with PCNB or PCA.
The incidence of abnormal fetuses per litter was significantly increased in the group of mice treated with the higher dose level of PCNB but not at the lower dose level and not with PCA treatment.
Malformations in the CD-1 mice were almost exclusively cleft palates.
The delineate if the teratogenic action of contaminated PCNB was due to HCB (see chapter on HCB), a purified PCNB sample was studied, at 500 mg/kg dose level, on days 7 - 16 of gestation.

The maternal liver to body weight ratio was not increased in contrast to the increases caused by the contaminated PCNB and the HCB.

There was an increased maternal mortality as well as a significant increase in fetal mortality and reduction in fetal weight.

The incidence of abnormal fetuses and cleft palates was comparable to that produced by the contaminated sample. In addition, there was a high incidence of clubfeet in these mice.

It is suggested that at least some of the teratogenic action of technical quintozene are due to the contaminant HCB. HCB produced cleft palates and some kidney malformations in OD-1-mice.

7. SPECIAL EFFECTS

a) Increased susceptibility of rats treated with PCNB to CCl_4 poisoning

Addition of 625 ppm of PCNB to the diet of rats for 25-26 days appeared to make rat more susceptible to the lethal effects of exposure to CCL_4 vapors (Finnegan, 1958).

b) Potential methemoglobin and Heinz Body Inducing capacity of PCNB in the cat (Schumann, 1976)

In view of its aromatic-nitro structure and reduction to pentachloroaniline as one of its metabolites, the methemoglobin generating potential of PCNB, its reversibility by methylene blue, and its ability to produce Heinz bodies were assessed. Adult female cats (four per dose) were orally intubated with PCNB as a 10% solution in corn oil (maximum solubility). Methemoglobin concentrations and Heinz body formation were assessed prior to, and 24, 48, 72 and 168 hr after the administration of PCNB. The percentage of circulating hemoglobin in the form of methomoglobin peaked from a preadministration value of 1.19% to a mean of 1.19% to a mean of 10.99 and 10.15%, 24 and 48 hr, respectively, after 1500 mg/kg of PCNB (p 0.05). Increasing the dose of PCNB to 2400 mg/kg failed to produce higher mean methemoglobin concentrations due probably to the emetic and cathartic effect of the large volume of corn oil administered. In a subsequent experiment, the intravenous administration of methylene blue (2 mg/kg) 40 hr after 1600 mg/kg of PCNB produced a decrease in mean methemoglobin values from 13.93 to 4.24% (p 0.05) in 1 hr. Both 1600 and 2400 mg/kg of PCNB produced a 10-fold increase in the number of circulating erythrocytes containing Heinz bodies. PCNB does not appear to be a potent methemoglobin inducer; that which is produced is reversed by methylene blue.

III OBSERVATIONS IN MAN

Skin irritation and sensitization in man (Finnegan, 1958).

Quintozene (75%) wettable powder) did not cause primary irritation when applied on a patch to the skin of 50 human subjects; however sensitization to a second application was produced in 13 of them.

No other data concerning the effects of PCNB on human were available to the reporter.

IV RESIDUES IN FOOD

Detailed data are available on the levels of quintozene residues in soils, plants, and animals.

The surveys have been summarized on a few monographs (FAO/WHO, 1970; FAO/WHO, 1973).

V CONCLUSIONS

- Metabolic studies performed on different animal species have demonstrated that PCNB does not accumulate in animal tissues and fat. It is rapidly metabolized into pentachloroaniline and methylpentachlorophenol sulfide, traces of which can be found in tissues of treated animals as well as in fetuses of mothers exposed to PCNB.

- Adequate acute as well as short-term toxicity studies demonstrate that PCNB has a low toxicity.

- A two-year study in rats has indicated a no-effect level of 25 ppm in the diet equivalent to 1.25 mg/kg.

- A similar study in dogs has indicated a no-effect level of 30 ppm in the diet.

The toxicological data have been considered sufficient for the establishment of a temporary ADI for man of 0.001 mg/kg body weight (FAO/WHO, 1974).

- The carcinogenic potential of quintozene has been evaluated by the oral route in two strains of mice. It produced an increased incidence of hepatomas in males of one strain and in females of the other one.
No epidemiological studies are available.

- Mutagenic studies have demonstrated that quintozene is non-mutagenic, either with the dominant lethal test or with microbial assay systems.

- Quintozene has been demonstrated teratogenic when administered at high dosage levels (500 mg/kg). to mice.
No teratogenic effects have been found in studies on rats.
The question as to whether the abnormalities are due to contaminating HCB is still debattable.

Additional studies are required in view of the establishment of dose/effect relationship criteria:

1. Studies to explain the sensitivity of dogs to the hepatic and bone marrow toxic effects of quintozene.
2. Additional studies on the toxicity of the main metabolites.
3. Comparison in rats, mice and dogs of the absorption, distribution and excretion of quintozene, its metabolites and contaminants of the technical product.
4. Carcinogenic studies in two additional animal species.
5. Additional studies on the teratogenicity of quintozene.

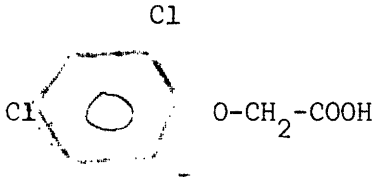
2,4-D and 2,4,5-T

2,4-D

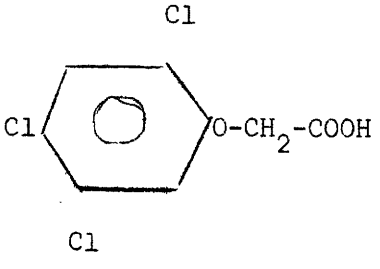
2,4-D is the common name for 2,4-dichlorophenoxyacetic acid.

2,4,5-T

2,4,5-T is the common name for 2,4,5-trichlorophenoxyacetic acid. Trade mark : Weedone 2,4,5-T.



2,4-D



2,4,5-T

I PHYSICOCHEMICAL PROPERTIES

2,4-D

Produced by the interaction of 2,4-dichlorophenol and sodium monochloroacetate; 2,4-D is a white powder of M.P. 240.5°C. Its solubility in water at 25°C is 620 ppm.

2,4-D, its salts and esters are systemic herbicides, widely used for the weeding of cereals and other crops at rates ranging from 280 g to 2.3 kg/ha, the highest rate persisting in soil for about one month. (British Crop Protection Council).

2,4,5-T

It is produced by the interaction of sodium monochloroacetate with 2,4,5-trichlorophenol. The latter may be made by the action of alkali on 1,2,4,5-tetrachlorobenzene produced by the chlorination of the trichlorobenzenes resulting from the dehydrochlorination of BHC (Galat, 1952).

The acid forms white crystals of m.p. 156.6°C; its solubility in water is 278 ppm at 25°C. Its salts with the alkali metals and amines are water soluble.

The technical acid is 95 to 98% pure, is stable an non-corrosive. The known impurities are in majority polychlorophenols. Apart from the presence of dioxins in polychlorophenols, heating of polychlorophenols will produce additional and very high yields of dioxin.

2,4,5 T resembles 2,4-D in its herbicidal properties and, though generally less phytotoxic, is more effective on woody species. It is used alone or with 2,4-D as a foliage, dormant shoot or basal bark spray, for girdling injection or cut stump treatment to kill woody plants. (British Crop Protection Council).

2. ACUTE TOXICITY

The oral LD_{50} have been determined on several animal species and summarized in tables I and II

II BIOLOGICAL DATA ON LABORATORY ANIMALS

1. METABOLIC STUDIES

a) Absorption, biotransformation and excretion

When administered orally as an amine salt, 2,4-D is rapidly absorbed in animals, concentrations in plasma reaching a peak after two hours for chickens, or four to seven hours for rats, calves and pigs. In the case of an ester of 2,4-D, absorption by the oral route is poor, as evidenced by low plasma and tissue levels following administration. The intact ester has not been detected in plasma or urine following treatment of rats, pigs or calves; only the free acid is found, a fact which indicates that hydrolysis occurs before absorption. Absorbed 2,4-D is distributed throughtout the body and passes the placental barrier as evidenced by experiments in pigs. The distribution pattern after administering an ester of 2,4-D is similar to that of the salts, altough tissue levels are lower. A partial binding to plasma proteins may occur. The biological half live in plasma for chickens, rats, calves and pigs was found to be 8,4, 8 and 12 hours, respectively. Elimination of 2,4-D when administred as an ester is less rapid than that of the salts. Excretion is mainly via the kidney, and only low levels have been found in the faeces and in the bile. Hens have been reported to excrete 2,4-D in their eggs, the compound occurring largely in the yolk (Erne, 1966 a et b).

After administration of ^{14}C-labelled 2,4-D most of the ^{14}C in the urine and tissues was present as unchanged 2,4-D, but minute amounts (0.25% of the dose) of an unidentified metabolite was found, particularly in the liver (Khanna and Fang, 1966).

This herbicide showed a remarkably reduced tendency to be conjugated, as compared with 2,4,5-T. Following oral administration of 2,4,5-T to rats and mice, the unchanged acid was found to be the main excretion product in the urine. The conjugates with glycine and taurine, as well as 2,4,5-trichlorophenol, were identified as metabolites. In rats, biotransformation of 2,4-D to the corresponding glycine and taurine conjugate was also detected. (Grunow, 1974).

The renal tubular transport in rabbits and rats by the organic anion mechanism may account for the relatively rapid disappearance fo both compounds, which in turn may contribute to their low toxicity (Berndt, 1973).

The half-lives values for the clearance of ^{14}C 2.4.5-T from the plasma of rats given doses of 5, 50, 100 or 200 mg/kg were 4.7, 4.2, 19.4 and 25.2 hr respectively. Urinary excretion of unchanged 2.4.5-T accounted for most of the ^{14}C activity eliminated from the body of rats. A small amount of unidentified metabolite was detected in the urine when rats were given 100 or 200 mg/kg but no 5 or 50 mg/kg. These results show that the distribution, metabolism and excretion of 2.4.5-T are markedly altered when large doses are administred (Piper, 1973).

In dogs given 5 mg/kg, the half-life values for clearance of plasma and elimination form the body were 77.0 and 86.6 hr, offering a plausible explanation of why 2.4.5-T is more toxic in dogs than in rats. Appreciable excretion in the faeces was noted and 3 unidentified metabolites were detected in urine of dogs, indicating a considerable difference in metabolism of 2.4.5-T in dogs and rats given the same dose (Piper, 1973).

Mice were injected with a single dose of 100 mg/kg body weight of 2.4.5-T in dimethylsulfoxide solution. The animals were sacrificed at various intervals after injection and analysed in toto. The amounts recovered as percentage of the amounts injected indicated decreasing levels at the following time intervals after dosing: at 0 hours, 77.1%, at 16 hours, 56.9% and at 24 hours, 23.7% (Zielinski and Fishbein, 1967).

Oral doses of 2.4.5-T and propylene glycol butyl esters of 2.4.5-T were given to cattle. Whithin 72 hr, approximately 86% of the administered dose was recovered from urine as the unmetabolized ester, and 1.4% was recovered as 2.4.5-T (Clark, 1971). St. John (1964) studied the fate of 2.4.5-T in cattle; it was eliminated as soluble salts in the urine, and no residue was found in the milk. Urine and milk samples were analysed from cows fed 2.4.5-T over a period fo four days; over 90% was recovered from the urine excreted over a period of six days.

Oral administration of a single dose of 5 mg/kg body weight of 2.4-D in a male human subject resulted in a plasma level of 35 ug/ml two hours after administration, it decreased slowly to 25 ug/ml after 24 hours and 3.5 ug/ml after 48 hours. Levels in whole blood decreased from 21 ug/ml

after two hours to 2.1 ug/ml after 48 hours. A total fof 73% of the dose was excreted in the urine within 48 hours following treatment. (Gehring and Gordon, 1971). Based upon this study it has been estimated that 1 mg/kg body-weight of 2.4-D can be eliminated by man within 24 hours.

Five human volunteers ingested a single dose of 5 mg/kg without occurring detectable clinical effects. Concentrations of 2.4.5-T in plasma and its excretion were measured at intervals after ingestion. The clearance of 2.4.5-T from the plasma as well as its excretion form the body occurred via apparent first-order rate processes with half-life of 23.10 and 23.06 hr respectively. Essentially all of the 2.4.5-T was absorded into the body and excreted unchanged in the urine. In the body, 65% of the 2.4.5-T resided in the plasma where 98.7% was bound reversibly to protein (Gehring 1973).

In a fatal poisoning in man, 2.4-D was found in blood, urine and tissues of all organs,, the brain had the lowest concentration - less than one fiftieth of that in the blood (Nielzen, 1965).

Absorption and urinary excretion of 2.4-D have been studied in six subjects following oral ingestion (5 mg/kg). 2.4-D is quickly absorded; significant quantities were detected in plasma after ingestion. 75% of the administered dose is excreted unchanged in the urine within 96 h after administration. Lack of metabolic transformation and rapid excretion suggest that cumulative toxicity by 2.4-D is unlikely (Kohli, 1974).

b) Biochemical effects

After administration of 2.4-D to rat (250 mg/kg), the synthesis of the acid-soluble organic phosphate is decreased. The study of the metabolism of these phosphate could explain that the lowered phosphorylating oxidation could be the pathogenic mechanism of intoxication by 2.4-D (Graff, 1972).

This confirmed the results of Heene and Rainer (1969) where rats received i.p. injection of 300 mg/kg 2-4-D and myophathy in the skeletal musculature became evident after 0.75 hr. This was correlated with a decrease of phosphorilase activity in the white muscle fibers.

In rat liver mitochondria, 2.4-D as been demonstrated to uncouple oxidative phosphorilation. At concentrations of 10^{-3} M, there was little effect on respiration yet the p:o ratio felt to 20% of the control level. Uncoupling effects were observed at levels as low as 5 x 10^{-5}M (Brody, 1952).

The effect of ingested phenoxy-acid herbicides on muscular function has been suggested to be related to interference with carbohydrate metabolism. Transitory diabetiform conditions have been reported in individuals spraying this compounds. However, hyperglycaemia or glycosurea could not be reproduced with certainty in rabbits; only one in five animals responded in this manner following administration of doses ranging from 125 to 500 mg/kg body-weight from 6 to 50 days. (Lorenzen, 1957), (Dalgaard-Mikkelsen, 1962).

2.4-D produced a dose related decrease in acetate metabolism in vivo in rats. A dose of 10 mg/kg body-weight of 2.4-D had some effect on carbon dioxide elimination form Na acetate (Phillco, 1967).

The nitrate content which increase in certain plants after spraying 2.4-D has been thought to be the cause of toxic effects which have been observed in grazing animals. Abortion in cattle have also been attributed to this phenomenon (Way, 1969).

In rats which were given 10 mg/kg body-weight of the sodium salt of 2.4-D by stomach tube for 120 days, the oxygen uptake decreased from 2.14 to 1.88 mm^3/mg of dry tissue in the heart, from 2.3 to 2.2 in the liver and from 5.7 to 2.32 in the kidneys (Stanosz, 1969).

Uptake of 131 Iodine by the thyroid in vivo is markedly increased by ingested 2.4-D (Florshein, 1962). It was demonstrated that 2.4-D lowers the serum protein bound iodine by lowering serum binding of thyroxine (Florshein, 1963).

table I

Acute toxicity of 2,4-D

Animal	Route	LD_{50} mg/kg body-weight	References
Chicken	oral	380-765 (amine salt)	Bjorn and Northen, 1948
Chicken (mixed)	oral	541	Rowe and Hymas, 1954
Mouse	oral	375	Hill and Carlisle, 1947
Mouse (M)	oral	368	Rowe and Hymas., 1954
Mouse	s.c.	280	Bucher, 1946
Rat	oral	666	Hill and Carlisle, 1947
Rat (M)	oral	375	Rowe and Hymas, 1954
Guinea pig	oral	1000	Hill and Carlisle, 1947
Guinea pig (mixed)	oral	469	Rowe and Hymas, 1954
Rabbit	oral	800	Hill and Carlisle, 1947
Dog	oral	100	Drill and Hiratzka, 1953
Dog	oral	541	Rowe and Hymas, 1954
Monkey	oral	428	Hill and Carlisle, 1947

table II

Acute toxicity of 2,4,5T

Animal	Route	LD_{50} MG/KG body-weight	References
Mouse	oral	389	Rowe and Hymas, 1954
Rat	oral	500	Rowe and Hymas, 1954
Guinea pig	oral	381	Rowe and Hymas, 1954
Dog	oral	100	Drill and Hiratzka, 1953

From data obtained with experimental animals, Vallet (1965) estimates that the lethal dose for man should be approximatively 50 to 500 mg/kg for 2,4-D and 500 to 1000 mg/kg for 2,4,5-T.

These speculations must, of course, be taken with very serious suspicion.

Monarca (1961) reported a case of a farmer who become ill after applying a 40 percent aqueous solution of 2.4-D by handpump against the wind.

A contaminant, 2.3.7.8-tetrachlorodibenzo-p-dioxin, causes serious acne in man and produced foetal deaths in hamsters at 9.1 ug/kg. Modern methods of manufacture of 2.4.6-T limit the amount of this contaminant to less than 0.5 ppm.

3. CHRONIC TOXICITY

Subacute toxicity tests have demonstrated that 2.4-D in large dose could produce poisoning in experimental animals and live stock. (Bucher, 1946, Hill, 1947, Bjorm, 1948, Drill, 1953, Rowe, 1954, Palmer, 1964).

One particular way of chronic toxicity for animals is from grazing posture sprayed at herb.cidal rates of 2.4-D or 2.4.5-T.

Mitchell (?1945), Dalgaard-Mikkelson (1959) and Goldstein (1960) all reported that there were no apparent ill-effect in cattle, sheep or horses from such grazing pasture sprayed with 2.4-D (herbicidal rate).

Attention, however has been drawn in the increase in nitrate content which has been observed in certain plants after spraying 2.4-D. Certain species reach a high nitrate content after heavy rain followed by preferential grazing of these by cattle. (Sund, 1960). A number of abortion in these cattle was correlated with occurrence of the high nitrate content.

Goldstein (1960) also reported spraying the skin of calf, cows,sheeps and of pigs with doses ranging from 0.002 to 0.008 pounds of 2.4-D or 2.4-D/2.4.5-T mixture, with no ill effects.

Dobson (1954) sprayed 2.4-D and 2.4.5.-T on grassed chicken runs daily for 14 days at normal and ten times normal dose rates. 2.4.5-T significantly reduced egg production and the weight of the birds. 2.4.-D mainly affected egg production in the second week of spraying. No effect on fertility were detected and all the progeny reared well.

When steers were given oral doses of 0, 50, 100, 200 or 250 mg/kg body-weight of an amine salt of 2.4-D for five days a week, poisoning was evident at 250 mg/kg after 15 treatments and at 100 mg/kg after 86 treatments. A dose of 50 mg/kg given 112 times had no effect on one steer (Palmer, 1963).

Bluegills were exposed to 0.1 - 10 ppm of the propyleneglycolbutyl ether ester of 2.4-D. About 20% of the fishes treated with 10 ppm died within eight days; mortality was almost negligible among bluegills exposed to 5 ppm or less. Pathological lesions were seen in the fish and the fishes treated with the high level had early and severe effects. The pathology involved liver, vascular system and brain, with depletion of liver glycogen,

globular deposits in the blood vessels and stasis and engorgement of the circulatory system in the brain (Cope, 1970).

It is reported that the butyl, butoxyethanol and propyleneglycolbutyl ether esters of 2.4-D are much toxic to fish than other 2.4-D formulations (Walker, 1964).

Most of study reported in the literature about toxicity of pesticides 2.4-D and 2.4.5.-T for animals showed that high doses were necessary to observe pathological effects.

- Guinea pigs tolerated 10 oral doses of 100 mg/kg body-weight of 2.4-D given over a 12 days period (Hill, 1974).
- When steers were given oral doses of 0, 50, 100, 200, 250 mg/kg of an amine salt of 2.4-D for five days, poisoning was evident at 250 mg/kg after 15 treatments and at 100 mg/kg after 86 treatments (Palmer, 1963).
- Rats fed a dietary level of 2.4-D for one month showed no adverse effects at 30 mg/kg (Hill, 1947).
- Mice which underwent daily injections of about 90 mg/kg body-weight of 2.4-D become pregnant and bore apparently normal litters at the end of a normal gestation period (Bucher, 1946).
- Strach et al (1964) reported that no abnormal behaviour in pig had been noted following 40 daily doses of 15 to 100 mg/kg of 2.4-D nor from single dose of 2.4-D of 200 to 800 mg/kg.

However, in short term trials by Bjorklung, 1966, calves and pigs showed definite though reversible symptoms of poisoning after single doses of 2.4-D of 200 and 100 mg/kg, respectively. Rats and fowls did not show any sign of distress after single doses of 100 and 300 mg/kg respectively, and fowls tolerated daily doses of 300 mg/kg daily in their food for several weeks without visible effects. Repeated daily doses of 50 mg/kg, however, led to toxic symptoms in some pigs.

In longer term studies (Erne, 1966a), five young pigs were fed 500 ppm of 2.4-D for up to 12 months but, although various toxic effects were noted and their growth rate was affected, none of the animals died.

Bjorklund (1966) administered 2.4-D in the drinking water of rats at 1000 ppm level. Progeny from treated females were maintened on the diets for 2 years with growth inhibition, poor general health and diarrhea as the main effects.

In the chronic toxicity study of Hansen (1971) 3 weeks old rats (male and female) were fed for two years 0, 5, 25, ... 1250 ppm 2.4-D in the diet. No significant effect on growth rate, survival rate, organ weight or haematological values was noted.

In the same study beagle dogs were fed 0,10, ... 500 ppm of 2.4-D in the diet for 2 yr, starting at 6 - 8 month of age. After this period no 2.4-D related effects were noted on the surviving dogs. Moderate depletion of cellular elements was noted at 10 ppm in 1 male that died after 10 months.

In a 3 generation - 6 litter rat reproduction study, no deleterious effect of dietary 2.4-D at 100 or 500 ppm was evident. At 1500 ppm however, 2.4-D, while apparently affecting neither fertility of either sex nor litter size, did sharply reduce the percent of pups born surviving to weaning and the weights of weanlings.

4. EMBRYOTOXIC AND TERATOGENIC EFFECTS

The teratogenicity of 2.4.5-T has been investigated and discuted mainly since the work of Courtney (1970). Their investigation indicated that the most significant malformations induced at high doses (4 mg/kg for rats and 46.4 mg/kg for mice and cystic kidneys in rats and mice. (Courtney, 1970).

The teratogenic effect was due no only to the 2.4.5-T but also to the high content (27 ppm) of the dioxin contaminant as confirmed in a later study (Courtney, 1971).

In fact, the study reported by Sparschu (1970) indicated that dioxin administered orally could produce fetal resorption and death in rats at levels as low as 0.5 ug/kg. It was concluded that the fetal effects could be the result of the dioxin contaminent.

The results of Emerson (1970) confirmed this when demonstrating that 2.4.5-T containing less than 1.0 ppm dioxin produced no abnormalities at doses up to 24 mg/kg.

However, using the purest sample of 2.4.5-T made available by Dow Chemical Co, teratogenic effects were induced in Swiss-Webster mice -

Cleft palates were noted at dose levels of 150 mg/kg and scattered abnormalities at 100 mg/kg.

Subsequent studies of Courtney (1971) showed that two relatively pure samples of 2.4.5-T (0.5 and 0.05 ppm dioxin) produced cleft palate and kidney malformations in mice at levels of 100 mg/kg or higher.

Other studies confirmed that the two phenoxyacetic acids at high doses are teratogenic and embryotoxic (Roll et al, 1971, (Neuber et al 1972).

Bäge et al, 1973 confirmed later that the most common defect was always isolated cleft palate - (50 and 110 mg/kg each acid). Less frequent were skeletal malformations and subcutaneous haemorrages. The skin of the living fetuses was very sensitive to handling with easily occurring subcutaneous bleeding.

Gastrointestinal bleeding has been detected after 2.4.5-T treatment of dioxin treatment on rat and hamster (Collins, 1971) (Courtney, 1971, Sparschu et al, 1971). Mixture of 2.4-D / 2.4.5.-T at the 110 mg/kg doses increased the frequency of renal heamorrhages.

Mrak (1969) indicated that 2.4.5-T was more teratogenic than 2.4-D. In fact in the study of Bäge et al, 1973, the foetal damaging effect of the combination of 2.4-D / 2.4.5-T did not exceed the effect of 2.4.5-T at a comparable dose of phenoxy acetic acid.

Further studies should be carried out to investigate a possible synergistic effect of the two phenoxy acteic acids and the interaction of dioxin contaminant.

Indeed there is a clear discrepancy between the teratogenic effect of 2.4.5-T containing 25 ppm dioxin, and the effects of the equivalent concentration of the same dioxin (Sparchu, 1971).

Courtney demonstrated also that the dioxin contaminant (TCDBD) alone produced cleft palate and a marked increase in the incidence of kidney abnormalities at levels of 1 ug/kg and 3 ug/kg. However when 100 mg/kg of 2.4.5-T was administred to mice with 1 ug/kg of TCDBD there was no increase in the rate of abnormalities.

Neubert (1972) showed that terata increased when levels of dioxin (TCDBD) in 2.4.5-T exceeded 1.5 ppm, and this was in agreement with Collins (1971) where the abnormalities per litter were related to the levels of the dioxin contaminant.

2.4.5-T or its Butyl ester (50 - 200 mg/kg) had specific teratogenic effects on foetuses when administred in a single oral dose to rats on the first to 16th day of gestation (Sokolik, 1973).

However, following a single oral dose of 2.4.5-T administered to a pregnant mouse or guinea pig, 50% of the dose is excreted within 15hr. This rapid rate prevent 2.4.5-T from reaching the foetus.

In the same manner foetal guinea pig injected in utero eliminated 2.4.5-T about as rapidly as the mother.

Lindquist (1971), suggested an interference with embryonic nutrition as the possible mecanism for the teratogenic action of the two herbicides These authors showed also a much more rapid disappearance from all embryonic tissues for 2.4-D and a stranger accumulation in the yolk sac placenta for 2.4.5-T.

The results of the study of the Bionetics Research Laboratories reported by Epstein (1972) showed again that the major abnormalities induced in mice were cleft palate and cystic kidneys, and in rats cystic kidneys and gastrointestinal haemorrhages. Increased fetal mortality was generally concomitant with these abnormalities. It is of particular interest than 39% abnormal embryos with cystic kidneys were seen in rats even at the lowest dose tested (4.6 mg/kg).

When given orally to sprague-Dowley rats at dosages of 4.6, 10 and 46.4 mg/kg from 10 to 15 days of gestation, 2.4.5-T produced an excessive fetal mortality, up to 60% at the highest dose and high incidence of abnormalities in the survivors was obtained. The incidence of fetuses kidney anormalities was therefold that of the controls even with the smallest dosage tested.

The Bionetics report (1969) concluded: "these results imply a hazard of teratogenisis in the case of this compound".

Moreover, the HEW Commission report (1969) clearly confirmed the original conclusions of the Bionetic report on the teratogenicity of 2.4.5-T.

In conclusion, the aforemen mentioned studies indicate that levels of nearly pure 2.4.5-T exceeding 100 mg/kg can be teratogenic in a number of animal species, and that in some species, the effect can be additive by increasing concentrations of the dioxin contaminant.

It should mentioned also, that two studies designed to evaluate the teratogenicity of nearly pure 2.4.5-T for the primate (Wilson, 1971) (Dougharty et al, 1975) indicated the compound non teratogenic at the dose levels given (40 mg/kg given three times/Wk, 10 mg/kg administered daily from day 22 though day 38 of gestation for Dougherty).

5. CARCINOGENICITY

There are only very few reports about carcinogenicity of phenoxy-acetic acids. In the study of Innes (1969), groups of 18 mice of each sex from two by hybrid strains were given 2.4-D from seven days of age for 18 months. The compound were given daily by gavage at levels of 0, 46.4 or 100 mg/kg body-weight until weanning, after with time 2.4-D was incorporated into the diet at the corresponding levels of 0, 149 and 443 ppm respectively. There was 10 significant increase between the controls and the groups given the two levels of 2.4-D.

In the long-term study of Hansen (1971) statistical analysis of randomly distributed tumor types indicated a tendency for the proportion of females with tumors to increase with 2.4-D dosage and a trend toward dose related increases of the proportion of malignant tumors.

III. OBSERVATION ON MAN

The effect of work exposure to 2.4.5.-T on the health has been studied. A total of 130 employeers having a work experience from two months to over three years were studied. No differences were found between the groups of man exposed to 2.4.5-T and a control group of 4600 men.

Some effects reported (Blerkey, 1964, Amon, 1970) should be related with some highly chlorinated phenolic ethers, and dioxins.

A man aged 23 comitted suicide by oral intake of 2.4-D. Pronounced degenerative changes of the ganglion cells of the brain were found upon histological examination (Nielsen, 1965).

Clinical symptoms of peripherical neuropathy have been reported in three individuals who had direct skin contact with on ester of 2.4-D.

General Conclusions

When the maximum human exposure to 2.4-D is compared to the dosage given the rats in most experiments, it is apparent that there is on extremely wide margin of safety between the 0.3 ppm, estimated daily intake and 1250 ppm (Highest level fed in the chronic study of Hansen, 1971). Indeed, if it is assumed that all the crops, for which a tolerance exists, always contained the maximun amount of 2.4-D permitted, it can be estimated that exposure to 2.4-D would be approximately 0.3 ppm (Hansen, 1971). This is the maximun daily intake proposed by the WHO/FAO (1971).

Moreover, following Gehring (1971) it has been estimated that 1 mg/kg body-weight of 2.4-D could be eliminated by man within 24 hours.

The importance of the dioxin contaminants has not been clearly defined. Several studies showed that 2.4-D or 2.4.5-T without any contamination could be toxic. The evidence so far presented is inconclusive to determine whether the teratogenic effects shown in mice and rats are due to the presence of a dioxin contaminant.

Moreover, the possibility of a synergistic effect should be reexamined.

Another unsolved problem is the observation that some of these compounds could induce porphyria (Poland, 1971). Wether prophyria is the result of the chemicals themselves or of the contaminants, or both, is not known.

Phorphyria could also be due to crude chlorobenzenes from which 2.4.5-T was made (Crow1970).

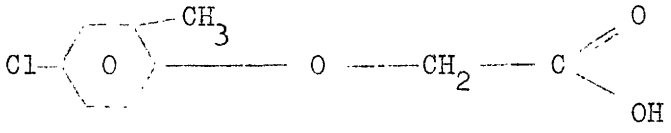
The long-term studies availables are for dog and rat. If we consider the large variation of toxicity between species, it could be necessary to extent the long-term study to other species and to reevaluate the ADI.

As we know that high dose levels of 2.4-D or salts produce embryotoxic effects, it should be interesting to see which salts are more embryotoxic, and what is the non-effect level, particulary for the butyl ester, as mentionned before by the WHO/FAO (1972).

In conclusion, if we know that the phenoxy-pesticides are rapidly eliminated by the man, and biotransformed by the plants, and that neither their toxicity, nor their carcinogenicity and teratogenicity seem to present a real risk, the conclusions of the FAO, 1971, may be taken into consideration until new informations are available.

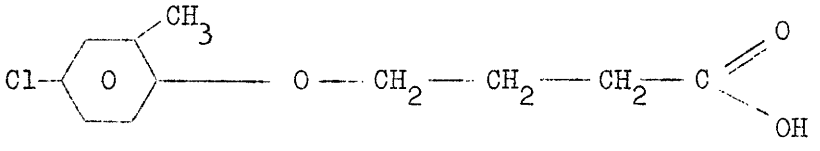
MCPA, MCPB, MECOMROP and DICHLORPROP

MCPA



MCPA is the common name for 4 - chloro-2-methylphenoxyacetic acid.
Commercial names: Agroxone, Agritox, Cornox M, Methoxon (Na salt) and Dikotex (Technical MCPA, diethanolamine salt).

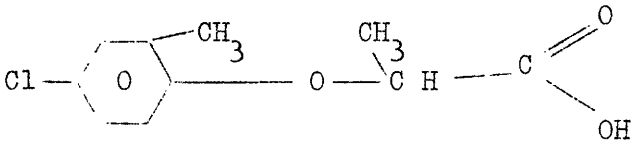
MCPB



MCPB is the common name for 4-(4-chloro-2-methyl-phenoxy)-butyric acid, also known as gamma-(4-chloro-o-tolyloxy)butyric acid.

Trade mark: Tropotox.

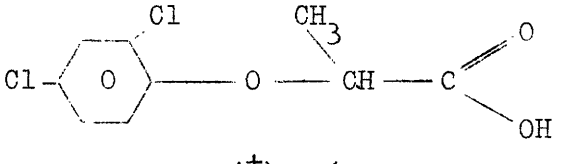
MECOPROP



Mecoprop is the common name for (±)-2-(4-Chloro-2-methylphenoxy) prppionic, acid or 2-(4-chloro-o-tolyloxy) propionic acid; the initials MCPP and CMPP have also been used.

Trade mark: Iso-Cornox.

DICHLORPROP



Dichlorprop is the common name for (±)-2-(2,4-dichlorophenoxy)propionic acid, also known as 2,4-DP.

Trade mark: Cornox RK....

I PHYSICOCHEMICAL PROPERTIES

MCPA

Made by the condensation of 4 chloro-o-cresol with sodium monochloroacetate in alkaline solution; the pure compound is a white crystalline solid of m.p. 118 - 119°C. As the chlorination of o-cresol gives a proportion of the isomeric 6-chloro-2 methylphenol, the crude MCPA contains the inactive 6-Chloro-2-methyl isomer, nowadays limited in amount. The crude production is of 85 to 95% purity and melts from 110 to 115°C and it is usual to quote the concentration of a particular product in terms of the active isomer.

The solubility of MCPA in water at room temperature is 825 ppm but it forms soluble salts of the alkali metals and organic bases, though these may be precipitated by hard water. Oil soluble esters may be prepared. The solutions of the alkali metals are alkaline in reaction and will corrode aluminium and zinc.

MCPA is a hormone-type herbicide readily absorbed by leaves and roots and translocated, used for the control of annual and perennial weeds in cereals grassland and turf at rates of 280 g to 2.25 kg a.e./ha. (British Crop Protection Council).

MCPB

Prepared by the condensation of chlorocresylate and butyrolactone the technical product of about 90% purity has melting point 99 to 100°C (pure: 100°C) Its solubility in water at room temperature is 44 ppm, in ethanol 15% and in acetone 20%. Its salts with alkali metals are water-soluble but precipitate by hard water.

MCPB owes its properties as a herbicide to the ability of susceptible plants to oxidise the acid to MCPA and it is used for the control of these plants in undersown cereals, peas and established grassland at 24 to 48 oz / acre.

Tropotox is an aqueous solution of the sodium salt containing 40% w/v MCPB. (British Crop Protection Council).

Residues may be determined by isotope dilution and infra-red spectroscopy (Glastonbury, 1959).

MECOPROP

Made by the condensation of alpha-chloropropionic acid with p-chloro-o-cresol, its contains equal amounts of two optical-isomers of which only the dextrorotatory form is active as a herbicide. Doses are usually expressed in terms of the mixture of isomers.

Mecoprop forms colourless, odourless crystals of m.p. 94 to 95°C. Its solubility in water at 20°C is 620 ppm but it is readily soluble in most organic solvents and forms water-soluble salts. The technical product may have a slight phenolic odour and melts at 90°C or above.

It is stable to heat and resistant to reduction, hydrolysis and atmospheric oxidation. It is corrosive to metals in the presence of moisture but solutions of the potassium salt do not corrode brass, iron or mild steel provided that the pH is not below 8.6 and the temperature less than 80°C.

Mecoprop is a translocatable post-emergence herbicide, recommended for the control of cleavers, chickweed and other weeds in cereal crops at rates of 1.5 to 2.5 kg/ha; it is also used in combination with other herbicides such as 2,4-D, MCPA, dichlorprop, ...(British Crop Protection Council).

DICHLORPROP

Dichlorprop may be synthetised either by the chlorination of alpha-phenoxypropionic acid, or by the condensation of alpha-chlorpropionic acid with 2,4 dichlorophenol. Having an asymmetric carbon atom, it exists as two optically-active forms of which only the dextrorotatory form is biologically active. The commercial product contains equal amounts of the two forms.

When pure, dichlorprop is a colorless, odourless crystalline solid of m.p. 117.5 to 118.1°C. Its solubility in water at 20°C is 350ppm but it is readily soluble in most organic solvents.

The acid is stable to heat and resistant to reduction, hydrolysis and atmospheric oxidation. Its potassium, sodium and diethanolamine salts have solubilities in water at 20°C of 90, 66 and 75 g acid equivalent/ 100 ml respectively. Salt formulations are compatible with similar formulations of other growth-active herbicides. The acid is corrosive to metals

in the presence of water but concentrated solutions (48% w/v acid equivalent) do not corrode iron or tin plate if the pH is 8.6 or higher and the temperature below 70°C.

Dichlorprop is a translocatable post-emergence herbicide effective in the control of weed in cereals at a rate of 2.5 kg a.e./ha and may be used either alone or in combination with other growth regulator herbicides such as 2,4-D, MCPA, mecoprop...

II BIOLOGICAL DATA ON LABORATORY ANIMALS

1) METABOLIC STUDIES

The following pathway for the microbial metabolism of MCPA is generally accepted:

MCPA → 6 hydroxy-MCPA → 5-chloro-3-methylcatechol → alpha methyl-gamma-chloro-muconic acid → α -methyl - γ-carboxymethylene → Δ - α-butenolide → α methylmaleylacetate → smaller fragments prior to entering a terminal respiratory cycle (Gannt et al, 1961)

MCPA is transformed to 2-methyl-4-chloro-5-hydroxyphenoxyacetic acid by Aspergillus Niger (Faulkner, 1965).

There are indication that MCPA is transformed by microorganism into 4-chloro-2-cresol (Audus, 1952).

The metabolism of these compounds are not well known in the mammals. Backe (1964) and St John (1964) studied the fate of MCPA and MCPB in cattle. All the MCPA fed to a single steer (113.5 mg single dose based on five ppm of a 50 pound daily ration) was accounted for in its urine over the four days after administration. No MCPB was found in the urine of MCPB-dosed cows (total dose of 56.75 mg and 113.5 mg based on 2.5 and 5.0 ppm of a 50 pound daily ration) on the first day after administration, but subsequent recoveries in their urine showed that 7.2 to 9.2% of the MCPB was converted to MCPA. Analysis of MCPB itself however was said to be unsatisfactory.

It seems that the phenoxyacids derivates were excreted nearly completely in the urine and were not or few metabolized.

2. ACUTE TOXICITY

From the acute orall and intraperitoneal LD_{50} values of MCPA and mecoprop in rats and mice (table 1), it is concluded that mecoprop is slightly less toxic than MCPA, and that on intraperitoneal injection the sodium salts are less toxic than the technical diethanolamine salts. The similarity between the intraperitoneal and oral toxicities for both MCPA and mecoprop indicates that both compounds are readily absorbed from the gastro-intestinal tract.

Table 1: Acute LD_{50} values (mg/kg) of mecoprop and MCPA in mice and rats.

Species	Route	Mecoprop		MCPA	
		Na	D	Na	D
Rat	Oral		1060 (±120)		800 (± 60)
Mouse	Oral	650	600 (± 35)	560	550 (± 30)
Rat	Intraperitoneal	500	350	400	300
Mouse	Intraperitoneal	600	400	500	350

Figures in parentheses represent ± SE.

Percutaneous tests with both compounds at levels of 800 -3200 mg/kg showed that they were poorly absorbed through the mouse skin, even when it was damaged (Gurd, 1965).

However, the DL_{50} values differed from author to author:

MCPA: oral, rats: 700 mg/kg (Rowe, 1954), 1010 mg/kg (D) (Faulkner, 1964).
Oral mouse: 540 mg/kg (D) and 590 mg/kg (Na) (" ")
Subcutaneous mouse: 462 mg/kg (D) and 640 mg/kg (Na) " ")
Cutaneous rabbits: more than 2 g MCPA / rabbit

Mecoprop: oral rats: 930 mg/kg (Woodford, 1965)

MCPB : oral rats: 680 mg/kg (" ")
700 - 1500 mg/kg

Dichlorprop: oral rats: 800 mg/kg
oral mice: 400 mg/kg Dermal mice: 1.400 mg/kg

3) CHRONIC TOXICITY

Oral chronic toxicity

Mecoprop and MCPA

Groups of 10 rats (5 males and 5 females) were given diets containing 0, 100, 400, 1000 and 2500 ppm of mecoprop (D) or MCPA (D) for 7 months. (A dietary level of 100 ppm for young rats is approximately equivalent to 10 mg/kg/day). The animals were weighed 3 times weekly, food consumption was recorded, and a haematological examination was made during the last 3 weeks of the study. On completion of treatment the animals were killed, the liver and kidneys weighed, and the following tissues taken for histological examination : heart, lung, liver, kidney, spleen, adrenal, stomach, ileum, pancreas, testis or ovary, thyroid, brain and femoral bone marrow.

Body weight gain

The weight gain in both male and female rats given dietary levels of 100 or 400 ppm of either compound was comparable to the controls. At the highest level of 2500 ppm, wieght gain was markedly reduced by both compounds in both sexes, especially during week 1-2. At the 1000 ppm level, the reduction in weight gain was less marked but still significant in all groups exept in the females on mecoprop.

Food consumption

Each group of 5 rats of the same sex receiving the same treatment was housed in one cage and the overall food consumption measured. These measurements were started 2 month before the test began and during this period animals consumed 13 - 20 g food/100 g body-weight with no consistent difference between the sexes. The only significant effect seen in the test was a reduction in the food intake by the males on 2500 ppm mecoprop during the first month. However, both males and females on 2500 ppm MCPA and males on 1000 ppm MCPA consumed considerably less food during wk 1 on test, then compensated for this in wk 2, so that food intake appears normal when averaged over the first month.

Haematology

Leucocyte counts, total and differential, were unaffected in any of the test groups. Erythrocyte counts, haemoglobin and packed cell volume were reduced by both compounds at levels of 400 ppm or more. These effects

were probably due to an increased rate of breakdown of red cells, as no histological abnormality of bone-marrow was manifest.

Mortality

A total of 13 animals died during the test or were killed because of obvious illness. Most of these deaths occurred in the groups given the highest dose of one or other compound. In most cases death was the result of infection, which suggests that maximum dosage of either compound led to a general reduction in resistance to infection.

Organ weights

The ratio of liver weight to body-weight in females was increased by both compounds at levels of 400 ppm or more, but that of males was significantly increased only by mecoprop at 2500 ppm. The relative kidney weight was increased in both sexes at all dose levels of both compounds.

Histopathology

The only significant histological change was seen in the livers of rats on 2500 ppm mecoprop. Nearly every animal in this group showed hepatic parenchymal cell enlargement with a homogeneous eosinophilic cytoplasm, sometimes involving a few cells at the center of the lobules or sometimes all cells one-half to two-thirds of the way through the lobules. These changes were not observed at lower dose levels of mecoprop, nor at any dose level of MCPA.

Organ weight recovery

A further experiment was carried out to study the reversibility of the increases in the relative liver and kidney weights observed in the pilot feeding study with mecoprop and in the 7 month feeding studies with mecoprop and MCPA.

Two groups of 60 rats (30 males and 30 females), aged 3 - 4 weeks, were given daily doses of 150 mg/kg of mecorpop (D) or MCPA (D) by oral intubation for 3 wk. In addition, two groups of 20 rats (10 males and 10 females) were given 60 mg/kg daily of each compound for the same period; appropriate control groups were also set up. After 3 wk of treatment all the animals given the lower dose of either compound, and a proportion of those on the higher dose and of the controls, were killed and their livers and kidneys weighed. No further treatment was given and remaining animals were killed 1 - 2 week later.

Rats on mecoprop (high dose) and MCPA (both levels) exhibited reduced weight gain immediately after treatment began, but all had made up the loss by the end of the 2-wk recovery period. Relative liver and kidney weights were increased by both compounds at the high dose level, but after recovery the relative liver weights were similar to those of the controls, except in the females on MCPA in which they were stillslightly elevated. The relative kidney weights remained significantly higher than those of the controls during the recovery period. No effect on relative liver or kidney weight was seen at the low dose level of either compound.

Conclusions

Over a period of 3 wk, daily oral doses of about 0.1 LD_{50} of either compound had little or no toxic effect in rats, while daily doses of about 0.25 LD_{50} brought about an increase in the relative weight of liver and kidneys. Liver enlargement was slightly greater in females than in males, but was reversible in both sexes within about 2weeks; the kidney enlargement was not reversible of discontinuing treatment.

Feeding experiments in rats lasting for 7 months again revealed little difference in toxicity between the two compounds. The main effects observed were increased relative weight of liver and kidneys, and anaemia; liver enlargement was much more striking in females than in males. There was a distinct reduction in weight gain at the 2500 ppm level, and a small reduction at 1000 ppm, with no effect on growth at lower levels. The only significant histological change was confined to most rats on 2500 ppm mecoprop and consisted of swollen liver cells.

It is concluded that mecoprop and MCPA are almost identical in their effects on the blood picture and organ weights when given in high doses to rats over periods up to 7 months. At dietary levels of 1000 ppm or less, the increase in relative liver and kidney weights was not accompanied by histopathological change, and at 100 ppm the only effect observed was slight kidney enlargement. These compounds thus appear to have a low degree of mammalian toxicity and are unlikely to present a hazard in normal use. (Gurd, 1965).

MCPA

A group of young rats was given diets containing 50 mg/kg pure methoxon (Na salt) and another groupe received technical dikotex (as 1% solution in water). Some rats died with signs of tiredness en a markedly reduction of body-weight. The other ones were not tired and the body-weight was normal. Any animals had myotonie. In the group receiving methoxon, the following histological changes were registered: in the lungs, some little parts of the epithelium were seen free in the bronchia, moderate degeneration of the parenchyme, congestia of the surrenals. In the group receiving dikotex oedematic changes with lung parenchyme bleeding and moderate degeneration of parenchyme were seen in the lungs. The central vain of the surrenals was congested; little bleeding of the parenchyme en moderate hyperaemia in the surrenal cortex were observed (Batora, 1957).

Young rats (around 150 g body-weight) were given diets containing a solution of 10% dikotex in water during one month. No local of general signs of disease were observed. The histological research showed local lung parenchyme necrose, specially localised in the lungs alveole. The surrenals were congested, with local bleeding in the parenchyme (Batora, 1957).

Four groups of 10 male and 10 female rats were given diets containing 0, 50, 400 and 3200 ppm of MCPA during 90 days. In the 3200 ppm group, the food consumption and the body weight gain were less than in the control group. In the first week of the experiment, the food consumption of the female 400 ppm group was lower than normal. The weight of the kidneys increase in the 3200 ppm and 400 ppm group. The females seem to have an increase of the brain weight.

Alcaline phosphatase, SGPT, hexobarbital oxydase, aminopyrine demethylase, aniline hydroxylase and the glucose -6 - phosphatase activity in the liver were the same than the controls. The histopathological research did not show any results attributable to the MCPA compound. In this experiment, the no-effect level was evaluated at 50 ppm.

Mecoprop

Groups of 6 rats (3 males and 3 females) were given daily oral doses of mecoprop (Na) for 3 wk at levels of 65 or 170 mg/kg. The animals were weighed daily, haematological examination was carried out on rats at the higher levels of dosage and on the controls, and a full autopsy was performed on all animals after the final dose. Growth retardation, enlarged kidneys and significantly increased liver weight (140% of controls) were observed at the highest level (Gurd, 1965).

4 groups of 10 males and 10 females rats received 0, 50, 400 and 3200 ppm MCPP in the diet during 90 days. In the 3200 ppm group the weight increased and the food uptake was lower than in the control group. The erythrocytes decreased from 400 ppm in the male group. A increase in liver weight was observed in 3200 ppm and lesser in the 400 ppm female group. The increase in kidney weight appeared in the 3200 ppm group, as well as the alkaline phosphatase. No histological changes were observed. In this experiment, 50 ppm was considered as the no-effect level.

Dichlorprop

No toxic effects were observed in rats fed 12.4 mg/kg/day for fourteen weeks though slight liver hypertrophy occurred at 50 mg/kg/day.

Dermal toxicity

MCPA

Groups of 5 rabbits received during 3 weeks 5 dermal application of MCPA at 0, 0.5, 1.0 and 2.0 g/kg levels. In the 1.0 and 2.0 g/kg groups, the body weight gain was less than in the control group. The skin was less elastic and erythematous . Some rabbits from the two higher group died, probably from infection, but the influence of MCPA can not discarded.

Mecoprop

Groups of 4 rabbits received, during 3 wk, 5 dermal application per week of 0, 0.5, 1.0 and 2.0 g MCPP/kg on the skin. The elasticity of the skin decreased in all groups. No significant differents were observed in blood analysis and in the organs controlled, but a decrease in the body weight was observed in the 0.5 and 1.0 g/kg group.

Toxicity of MCPA for cows

Cows tolerated 30 mg/kg/day MCPA for 21 days without detectable symptoms (Dalgaard, Mikkelsen, 1959).

4. EMBRYOTOXIC EFFECTS

Five pregnant rats, which have had youngs in the past, received 150 mg/kg MCPA orally by intubation. MCPA was administered during the 10 - 11th day of pregnancy. 3 rats were delivered of 2, 7 and 10 youngs, after the normal time of pregnancy. The two other nests were lost by cannibalism. It seems that there was no influence of MCPA on the lenght of pregnancy.

Dobson (1954) sprayed MCPA on grassed chicken runs daily for 14 days at normal and ten times normal dose rates. MCPA affected eggs production mainly in the second week of spraying or during the week after spraying had stopped. But there was no effects on the fertility of the eggs and all the progeny reared well.

Dunachie and Fletcher (1967) injected hen's eggs with MCPA and with MCPB. Dose rates were 10, 100 and 200 ppm, equivalent to 0.5, 5 and 10 mg/egg. The percentage hatch was recorded. At the lowest dose there was 100 % hatch from the MCPA and the MCPB treated eggs. At the higher dose, there was 20 % hatch from the MCPA and MCPB treated eggs. None of the chicken that hatched was deformed although some feather blanching was noted from the treatment.

Dunachie and Fletcher (1967) injected chicken's eggs with mecoprop. Doses were 0.5, 5 and 10 mg MCPP/egg. At the lowest dose, the hatching was 90%. At the higher dose, 80% hatching.

III BIOLOGICAL DATA ON HUMANS

The lethal dose (LD_{50}) for MCPA in rats is about 700 mg/kg (Rowe, 1954). There is considerable variation in species susceptibility and dogs appear to be particularly vulnerable to chlorinated phenoxy-acids and derivates.

Within a few hours of administration, the animals (dogs for Dalgaard 1962) develop muscular weakness and ataxia followed by rigidity clonic spasms and coma. True convulsions did not occurr, although opisthotonos has been reported. Anorexia and vomiting are prominent and are frequently accompagnied by bleeding from the nose and mouth and bloody diarrhea. Leucopenia and thrombocytopenia are occasional but inconstant findings. In rats EEG changes consisting of high voltage delta waves have been noted (Desi, 1962) as well as disturbance of thyroid function (increased ^{131}I - Iodine 136 uptake and reduced serum protein - bound iodine) (Florshein, 1962 and 1963). Autopsy of experimental animals may reveal ulcers of the oral mucosa and necrosis of small intestinal mucous membrane. Renal and hepatic changes are characteristic and consist of cloudy swelling of the proximal renal tubules and centrilobules and necrosis of parenchymal liver cells (Hill, 1947).

In man, our knowledge of the DL_{50} and toxic effects of these com= pounds are incomplete. Fatal cases of poisoning have occurred with doses of 14 g MCPA (Hohnson, 1965). In another case, 100 g MCPA (1.9 g/kg) was absorbed. In fact, the patient probably vomited shortly after drinking and the absorbed dose was probably much less than 100 g (Jones, 1967) (T.2)

Table 2: Recorded Cases of suicidal poisoning with chlorinated phenoxy acids

		Approximate dose		patient		
Author	Substance	total (gr)	mg/kg	age	sex	Outcome
Popham (1964)	MCPA	22	440	32	M	Died
Johnson (1965)	MCPA	14	250	65	M	Died
Jones (1966)	MCPA	100 or less	1.900	61	M	Recovered

In man, the features of acute intoxication are similar to those re- ported in animals. Vomiting may occur; neurological signs include coma,

clonic muscle spasms, fibrillary twitching, and constricted pupils. Tendon reflexes may be diminished. In one case opisthotonos and major convulsions occurred (Popham, 1964). The impurities present in the product may contribute to the clinical picture. Popham et Davies (1964) observed venous congestion of the liver with areas of fatty necrosis while Curry (1962) commented on degeneration of the renal tubules. Nielsen (1965) reported severe degenerative changes in ganglion cells of the CNS, but these may have been secondary to anoxia. There is no antidote and treatment is entirely supportive.

IV CONCLUSIONS

The principal routes of toxicity to man are either orally or by inhalation. There appears to be little hazard of transport through the skin although individual allergies can develop leading to dermatitis (Vallet, 1965). Eyes may be directly but usually only temporarily affected. Hazards to man may occur from the concentrated chemical before dilution, from inhalation of spray or dust during application, or from ingestion of the chemical in food or in water.

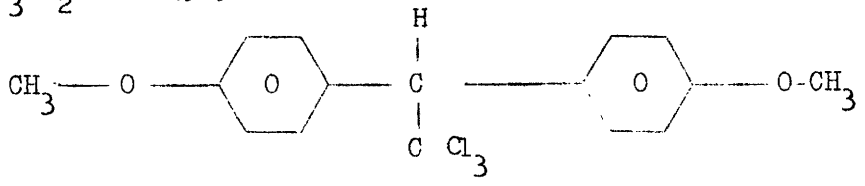
It is generally accepted that auxin-type herbicides do not present a direct toxicity hazard to man (Barnes, 1965).

Vallet (1965) estimates the acute toxic oral dose for MCPA at a level of 50 mg/kg, as extrapolated from data on laboratory animals. Freireich (1966) has shown that a number of drugs, on a mg/kg basis, are 10 to 15 times more toxic in man than in the mouse. A very much more accurate assessment of the ratio of animal to human toxicity can be obtained on a mg/m^2 of body surface area rather than on a mg/kg basis.

Duggan (1967) calculated pesticide chemical residues in food. MCPA was found in grain and cereals, and the daily intake was estimated at 0.002 mg (in 1965-1966).

METHOXYCHLOR

$C_{16} H_{15} Cl_3 O_2$ M.W 345.5.



1,1,1, trichloro - 2,2, - di - (4methoxyphenyl) ethane or
1,1,1,-trichloro - 2,2 - bis (p- methoxyphenyl)ethane
dimethoxy - DT, dimethoxy-DDT, methoxy DDT
Marlate, moxic,
DMDT, di (p-methoxyphenyl)-trichloromethyl methane,
dianisyltrichloro-ethane 2,2-di-4-anisyl, 1,1,1-trichloroethane.

Insecticide: it is a non systemic contact and stomach insecticide with little aphicidal or acaricidal activity. It is generally non-phytotoxic.

Physico chemical properties

The technical products contains about 88% of the p,p'isomer, the bulk of the remainder being the o,p, isomer. It is a grey flaky powder of $d1^{25}$ 1.4, practically insoluble in water, moderately soluble in ethanol and petroleum oils, readily soluble in most aromatic solvents. The pure p,p' isomer forms colourless crystals of m.p. 89°C. It is resistant to heat and oxidation and it is less readily dehydrochlorinated than DDT by alcoholic alkali. It is susceptible to dehydrochlorination by heavy metals catalysts.

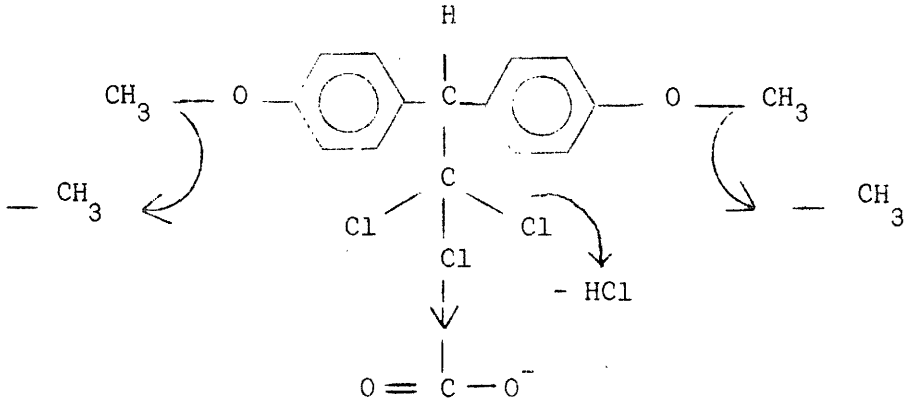
1) BIOLOGICAL DATA ON LABORATORY ANIMALS

Metabolic studies

The only available informations concerning the methoxychlor relate to its metabolism in rats and mice plus a very few data on the urinary excretion in rabbit (FAO PL 1965/10/1 - IARC Monographs vol 5 1974 and Smith 1946).

Methoxychlor is not excreted intact, its metabolism takes place in the liver which very rapidly detoxicates the insecticide and excretes its metabolites throughout the bile via the intestine in the faeces (van Oettingen, 1946). In mice as much as 98.3% of the oral dose of labelled

methoxychlor are excreted in 24 h. In rat, fecal excretion exceeds urinary excretion and 40% of a i.v. dose is excreted in the bile in 6 hr (Weikel, 1957). During its liver metabolism, methoxychlor undergoes dehydrochlorination in the trichloroethane group forming an ethylene bound. It suffers a sequence of reactions involving dechlorination, dehydrochlorination and reduction, followed by hydration and oxidation to form the respective disubstituted acetic acid. In addition at undergoes O-demethylation, (Lykken, 1969).



The gastro-intestinal absorption of both methoxychlor and its metabolites is low and there is no entero-hepatic recirculation (Weikel, 1957).

As compared to DDT the storage of methoxychlor in tissues or fat is rather low but it definitely takes place. Given in diet at doses of 500 ppm or 100 ppm methoxychlor accumulates in fats (respectively 14-36 ppm and 1-7 ppm). This accumulation reaches a maximum after 4 weeks and the stored material is mobilized in 2 to 4 weeks after exposure (Kunze 1950). At a dose of 25 ppm in diet there is no storage. An excretion in the milk has been reported for methoxychlor, this excretion is proportional to the dosage.

2. ACUTE TOXICITY

The methoxy analogue of DDT, DMDT is of a very low order of toxicity. At a dose of 7 g/kg p.o. to the rats, it causes diarrhea, progressive weakness and death of 53.8 % of the animals in 36 - 48 hr. (Smith, 1947).

Depending of the species, the LD_{50} is between 1850 and 700 mg/kg of body weight (FAO PL 1965/10/1).

Animal	Route	LD_{50} mg/kg b.w.
Mouse	Oral	1850
Rat	Oral	5.000 to 7.000
Sheep	Oral	> 2000

3. CHRONIC TOXICITY

Rat

Rats were fed for 45days on a ration containing 100, 1000 and 30.000 ppm methoxychlor. At 100 ppm there was no effect on growth; at 1000 ppm growth was slightly retarded; at 30.000 ppm very little growth occurred. There was no deaths in the 100 ppm and 1000 ppm groups; 80 % of the rats died in the groups receiving 30.000 ppm. The blood picture was normal. At autopsy there was no significant difference in the organ-weights of the rats on 100 ppm and 1000 ppm. The 30.000 ppm group showed uniformly smaller organ-weights than the controls. In the case of the testes, the decrease in weight was very marked. There was no evidence of histopathological change in the organs examined, except in testes, which showed apparent suppression of spermatogenesis beyond the spermatogonal phase. (Hodge, 1950).

When rats were fed for 2 years on diets containing 25, 200, 1000 ppm of methoxychlor, there was no effect on growth at doses of 25 ppm and 200 ppm, but there was moderate reduction in growth at 1000 ppm. There was no decrease in life span; organ-weights and blood picture were essentially normal and histopathological examination revealed no significant changes (Hodge, 1952).

Rabbit

Daily oral doses of 200 mg/kg b.w. killed the rabbits after 4 to 15 days, The only symptoms noted were diarrhoea and anorexia (Smith, 1946).

Dermal application of 2 or 3 ml of a 30 % solution in dimethyl phtalate for 13 weeks was toxic; growth was depressed and paralysis of the foreleg occurred in some cases. Histopathological examination showed some

fatty degeneration of the liver and lesion of the central nervous system. Application of 1 ml or less had no effect (Haag, 1950).

Dog

Dogs were maintained for one year on doses of 20, 100 and 300 mg/kg/day. There was no death, both blood picture and organ-weights were normal and there was no histopathological modification (Hodge, 1952).

4. CARCINOGENIC EFFECTS

Methoxychlor was tested by the oral route only in the rat. Three experiments, including one employing dietary levels of up to 1000 ppm (equivalent to about 80 mg/b-w/day), provided no evidence of carcinogenicity. Because of inadequate reporting, conclusions cannot be drawn from the results of a fourth experiments in which some liver tumours were observed in rats fed up to 200 ppm in the diet (equivalent to about 100 mg/kg/day). Data from these four experiments do not allow an evaluation of the carcinogenicity of methoxychlor to be made at the present time.

No tumours were reported in limited skin application (10 mg in 0.2 ml acetone weekly to about 400 days) and subcutaneous injection (10 mg in 0.02 ml trioetamoin) studies. (IARC monographs Vol 5 , 1974).

5. MUTAGENIC EFFECTS

No reported information.

6. EMBRYOTOXIC EFFECTS

It has been demonstrated repeatedly in nature that the neonatal individual is extremely sensitive to insecticides.

Mortality in the newborn obviously can be elicited at insecticide concentrations far below that causing death in an adult.

The placenta offers no effective physical barrier to the passage of chorinated hydrocarbon insecticides.

DDT readily crosses the placenta of the rabbit, the mouse, the dog, the rat and the human (Ecobichon, 1970). There is however no reported data concerning the effects of DDT on the foetus or the neonate.

7. SPECIAL EFFECTS

Rats fed 30.000 ppm of methoxychlor in diet for 45 days showed a marked reduction in testes-weights. These testes showed apparent suppression of spermatogenesis beyond the spermatogonial phase; the spermatogonia and Sertoli cells were relatively normal; the primary spermatocytes were variable in number, usually with evidence of necrosis. The more mature germ cells were absent (Hodge, 1950).

In paired-feeding experiments in which 10.000 ppm of methoxychlor were added to the diet of weanling male rats, a marked reduction in the weight of the testes, vesicles and prostate was found (Tullner, 1962)

These effects could be mediated through an oestogenic action of methoxychlor inhibiting the production of anterior pituirary gonadotrophins with consequent deficiencies in the development of the male reproduction system.

Several investigates have indeed suggested an estrogenic activity for the chlorinated insecticide DDT and its analogs in various animal species.

Foster howevere reported that consumption of methoxychlor by chickens at levels as high as 10 ppm produced no significant estrogenic effects. Moreover, no toxic effects occurred over a 5 week feeding period at this level (Foster, 1973).

III BIOLOGICAL DATA ON HUMAN

They are very few informations concerning the potential effects of methoxychlor on human. The estimated fatal oral dose for man is approximately 6 g/kg b-w. An oral dose of 350 mg/kg/man/a day for 2 years produced no symptoms whereas 500 mg/man/day produced tissue changes (American Conference Ohio, 1974).

IARC (1974) reported that no epidemiological studies were available to the working group, concerning the potential carcinogenic effects of methoxychlor.

Human chang liver and Hela cells were exposed to organophosphorus, organochlorine, and dinitrophenol insecticidal compounds. The acute cytotoxicity was measured during 48 hr incubation and the effects were expressed in two ways: the toxic dose (TD50) based on cytopathological effects observed microscopically and the growth inhibition (ID50) based on inhibition of cell protein systhesis.

The TD50 for methoxychlor was very high and equal to $\pm$ 1 mg/ml. (Gablicks, 1965). It appeared to be the less cytotoxic organochlorine compound and even the less cytotoxic of all the tested insecticides.

IV RESIDUES IN FOOD

Due to its rapid degradation (Metclaf, 1970) and low tissue storage (Weikel, 1957) methoxychlor is found in only trace amounts in food items.

Very little methoxychlor is excreted in the milk of cows consuming feed contaminated with this insecticide. It has perhaps the lowest propensity among the commonly usual organochlorine insecticides to be excreted in the milk. Relative excretion rates of aldrin, dieldrin, DDT and heptachlor in milk are 1685, 832, 107 and 88 respectively, taking methoxychlor as unity (Saha, 1969).

In total diet studies carried out in the U.S. in 1963-1969, only traces of methoxychlor were detected in dairy products (Duggan 1971) while in another study in 1969-1970 the concentration ranged from 0.008-0.059 ppm (Corneliusson, 1972). No methoxychlor was detected in a U.K. total diet study (Abbott, 1969).

V CONCLUSIONS

Methoxychlor is rapidly metabolized mostly in the liver, it is quickly excreted throughout the facces via the bile and it appears also in the urine.

Methoxychlor appears to be stored in the fat but this storage is low as compared to DDT.

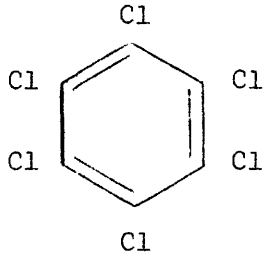
Its LD_{50} is relatively high in all animal species studied. It induces no specific toxic effects except perhaps an estrogenic like modification of the testes. Rats appear to be the most sensitive animal species. There are no reported carcinogenic effects in animals. There are no reported indications concerning the potential mutagenic and teratogenic effects of methoxychlor. The effects on human are very poorly documented even of a report showed its low cytotoxic effects on human isolated liver cells.

Due to its low level in food, the total dietary intake of methoxychlor is relatively very low. In the U.S., it ranges from 0 in 1965 to traces in 1966, 1969 and to 0,02 ug/kg/b-w/day in 1967, 1968, 1970 (Duggan, 1972).

According to FAO (FAO PL 1965/10/1), methoxychlor is thus apparently a relatively safe organochlorine insecticide as compared to the other chemical compounds. Its use however could not be extended before evaluating its teratogenic and mutagenic potencies and before reexamining its carcinogenic effects.

Based on what has been reported until now, the estimate of acceptable daily intake for man has been set up at 0 to 0.10 mg/kg body-weight. (FAO PL 1965/10/1)

HEXACHLOROBENZENE



1,2,3,4,5,6 hexachlorobenzene

Synonyms : HCB, perchlorobenzene.

Hexachlorobenzene should not be confused with the pesticide benzene hexachloride.

Uses

Hexachlorobenzene is a fungicide. It is used as dusts containing 10 - 40 % HCB alone or together with small quantities of lindane (0,5 - 1 %) added to prevent insect attack on stored seed.

One of the current uses of HCB is in the treatment of seed such as that of barley, flax, rye, wheat, corn, peanats and onions.

In as much as HCB is also a by product of chlorine gas and chlorinated hydrocarbon production and because it is used in the manufacture of pentachlorophenol, it seems highly probable that HCB enters the environment from industrial sources.

Hexachlorobenzene also exists as a contaminant of pentachloronitrobenzene a soil fungicide used in the production of cotton and other crops (Booth N.H 1975).

I. PHYSICO CHEMICAL PROPERTIES

HCB is a colourless white powder or needless crystal. Its melting point(MP) is 229°C and its boiling point (BP) 326°C.

It has a vapor pressure of 1.09 10^{-5} mmHgat 20°C and it is sublimable.

HCB is insoluble in water and alcohol. It is soluble in hot benzene.

The technical grade used in agriculture contains 98 % hexachlorobenzene, 1.8 % pentachlorobenzene together with 0.2 % 1,2,4,5 tetrachlorobenzene (FAO, 1970).

II . BIOLOGICAL STUDIES ON LABORATORY ANIMALS

1. METABOLIC STUDIES

Parke and Williams (1960) gave HCB to rabbits as an aqueous suspension and found absorption from the gut to be 4% at most.

It has been found in rats (Koss, 1975) that intestinal absorption of HCB from an aqueous suspension is very poor and that the amount of HCB left in the intestinal contents 24 hr after administration was small.

When the compound is given in a solution of oil at doses of 20, 60, and 180 mg/kg the intestinal absorption averaged 80% of the dose.

The fact that HCB is well absorbed when dissolved in oil is of particular relevance to the problem of HCB residues in human and animal food. If one presumes that HCB in food is dissolved in lipids its absorption from the contaminated food should be considered to be far better than absorption from a suspension of the fungicide crystals in water (Koss, 1975).

The absorbed HCB is distributed in all tissues; it accumulates in fat and skin but it is also stored in the liver, brain, kidney and blood. Its concentration in the muscle is rather low while its concentration in adrenals and ovaries is as high as that of skin.

From day 2 onwards, content of radioactivity declined gradually in the various tissues. The slope of this decline was parallel in all tissues and was independent of the dose and the route of application. The half life of radioactivity was about 8 to 10 days. In most tissues examined, the radioactivity was shown to be almost exclusively due to unchanged HCB.

The analysis of feces and urine demonstrated that HCB was subject to biotransformation since only a portion of the radioactivity was excreted in form of the unchanged fungicide. The possible metabolites in urine and feces have not yet been identified but it has been reported (Parke 1960) that HCB did not form conjugated glucuronic acids, ethereal sulphates or mercapturic acids when administered orally to rabbits at a dose of 400 mg/kg.

Using rats treated either by phenobarbital or carbon tetrachloride, no direct correlation could be made between the induction or the inhibition of microsomal enzyme activity and an increased or a decreased

rat e of disappearance of HCB residues in liver tissue (Villeneuve, 1974).

There is evidence that HCB (or a toxic metabolite of it) is excreted in milk of rabbits (De Matheis, 1961) and many reports have demonstrated that human milk from various countries contained HCB (Luquet, F.M., 1972, 1975, Siyali, 1973, Miller, 1973).

2. ACUTE TOXICITY

LD_{50} (FAO, 1970) (Oral)

Animal	LD_{50} mg/kg/b-w
Mouse	4000
Rat	3500
Guinea pig	> 1000
Rabbit	2600
Cat	1700

500 mg/kg ip is mon lethal in rats; the oral lethal dose in guinea pig is greater than 3 g/kg; the oral lethal dose of a 15% suspension of HCB in female Japanese Quail is greater than 1 g/kg (Vos, 1971).

3. CHRONIC TOXICITY : (Short-term)

Chicken

HCB given at levels of 120 - 480 ppm in the diet for 3 months caused no toxic effects in chicken (Melis, 1955).

Japanese Quail: A preliminary subacute toxicity study in Japanese Quail demonstrated that the feeding of 20 ppm HCB for 3 months was still toxic (Vos, 1968) by causing disturbance in porphyrin metabolism and diminished reproduction.

In an other study (Vos, 1971) HCB was given for 90 days to Japanese Quail at dietary concentration of 0, 1, 5, 10 and 80 ppm. Tremors and mortality occurred in birds fed 80 ppm. Red fluorescence of tissues (typical of porphyria), liver damage (enlargement of nuclei and nucleoli, proliferation of bile ductules, necrosis of hepatocytes), erythophagocytosis in the spleen (suggestive of a higher tumover rate of red blood cells)

ceroid granules in the tubules of the kidney, reduced reproduction and reduced volume of eggs were found in birds from the 80 ppm group.

Increased liver weight, slight liver damage and enlarged fecal excretion of coproporphyrin occurred in the 5 ppm group; the no-effect level was established at 1 ppm.

Rat

Ad libitum feeding to rats of diets containing 0.2% HCB resulted in the death of 40% of the rats within a month. (Ockner, 1961). Before death, the rats developed tremors, ataxia, weakness, and paralysis, without evidence of major disturbances in porphyrin metabolism. In the remaining rats, an increase in the urinary excretion of porphyrins and porphyrin precursors was detected after 2 to 8 weeks of HCB administration. Hepatomegaly was a common finding in the rats, and degeneration of the hepatic cells was observed histologically. Grossly an intense red fluorescence due to the presence of porphyrin was observed in the cortex but not in the marrow of long bones.

Additionally, skin lesions in the form of depilated sores with hemorrhagic crusts were reported (De Matteis, 1961). The lesions appeared before the development of porphyrinuria, most often near each sgoulder.These lesions may have developped as a result of the rats' scratching in as much as signs of itching were observed. It was suggested that the lesions resulted from chronic dermal initiation rather than from photosensitivity. In studies made by other investigators (Pearson, 1965) however, photosensitization could be induced in the rat by long-term, continuous exposure to UV light and by feeding 1.000 to 4.000 ppm of HCB for 3 to 4 months.

In a study in which Sprague - Dawley rats were fed 1 to 100 mg of HCB/kg b.w./day for 30 days, examination of the tissues did not show any definitive pathologie changes at dosages of 1, 3 and 10 mg/kg/day (Booth, 1975). At dosages of 30, 65 and 100 mg/kg/day, however, there were gross and histopathologic alterations involving the liver. Thus, no-effect dosage for 30 day exposure would seem to be 10 mg/kg/day. In long-term studies, the no-effect dosage seems to decrease as the exposure period increases. For example, an effect from as little as 0.1 ppm of HCB in the drinking water was noticed in rats after an exposure of 120 to 140 days: the daily dosage of HCB was 0.025 mg/kg/bw.

Two cases of HCB toxicosis have been reported in pregnant rats (De matteis, 1961). On of the rats died after consuming a diet containing 5.000 ppm HCB; the period of exposure was not reported. The remaining rat delivered normally and reared its young until they died after convulsive seizures, which appeared when the offspring were 7 to 8 days old. The dam, in turn, was given 3 normal week-old infant rats to foster. These rats died 3 to 4 days later after convulsive seizures developped. This evidence indicates that HCB is excreted in the milk and that newborn rats are extremely sensitive to the effects of HCB.Newborn animals of other species may also be equally sensitive.

Mouse, Guinea pig, Rabbit and Cat

Other small laboratory animals develop signs of HCB toxicosis similar to those described for the rat, but the mouse was found to be particularly susceptible, neurologic signs developping within 8 to 10 days following the feeding of 0.5 % HCB in the diet (De Matteis, 1961).

In the guinea pig, the neurologic signs were similar to those of other laboratory animals (De Matteis, 1961). Toxic effects were not observed when guinea pigs were given 1 g of HCB / kg b.w. p.o in a single dose or after they were given 120 ppm in the diet daily for 3 months (Melis, 1955) of all the laboratory animals studied, the biochemical changes associated with HCB toxicosis in the rabbit most nearly ressemble those described in man (De Matteis, 1961).

Mouse, guinea pig and rabbit all develop striking neurologic signs after exposure to HCB. The nervous signs of HCB toxicosis do not seem to be closely related to disturbed porphyrin metabolism because they develop rapidly and before pigment metabolism becomes affected. Consequently, porphirinuria must be regarded as a chronic effect of continued HCB intoxication, not as a feature of toxicosis in animal dying earlier from neurologic complications (De Matteis, 1961).

4. CARCINOGENIC EFFECTS

No information has been found concerning any carcinogenic action.

HGB however has been reported to induce the biphenyl-2-hydroxylase as do some carcinogens (Turner, 1974).

Furthermore pentachloronitrobenzene (PCNB) has been reported to be tumorigenic in mice at a dose of 464 mg/kg/day (Innes, 1969), and it is known that PCNB is often contaminated with HCB which has been reported to be, at least partially, responsable for the embryotoxicity of PCNB (Courtney, 1976).

5. MUTAGENIC EFFECTS

Except for one report showing no changes in fertility of male rats dosed with 60 mg/kg daily for 10 days (Klera, 1974) there is no information concerning the mutagenicity of HCB.

6. EMBRYOTOXIC EFFECTS

Hexachlorobenzene crosses the placenta and accumulates in the foetus in a dose dependent manner. The fetal liver seems to accumulate HCB. Neither in rabbit nor in rat was it any evidence of fetopathic effects. The doses of HCB were 0, 0.1, 1.0 or 10 mg/kg p.o daily from day 1 to day 27 of gestation for the rabbits and 5, 10, 20, 80 and 120 mg/kg p.o daily from day 6 to day 16 of pregnancy for the rats (Villeneuve, 1975, 1974).

In an other study in rats reported by Khera (1974) with HCB doses that induced no apparent maternal toxicity (10, 20, 40, 60 mg/kg/day during days6-9, 10-13, 6-16 or 6-21 of gestation) the only positive teratogenic findings were increases in the incidence of 14th ribs and sternal defects. But since these defects were not reproducible in subsequent trials at doses up to 80 mg/kg given during organogenesis, their significance in attributing a teratogenic potential to HCB is doubtful. It must be recalled however that the extrarib has been considered as an indicator of teratogenic potency (Kimmel, 1973, Yasuda, 1972).

In a recent study, however, (Courtney, 1976) it is shown that mice of the CD-1 strain treated with HCB had significantly increased maternal liver to body weight ratios and decreased fetal body weights. In addition, there was a significant increase in the incidence of abnormal fetuses per litter. Some cleft palates were produced and they all occurred in one litter. The interesting observation was that the few kidneys affected were mainly of small size and there was one case of renal agnesis. From this study it can thus be concluded that the possibility exists that HCB could be a teratogenic agent in the mice.

It is however readily apparent that a great deal more needs to be known about HCB and its effects on fetal devdopment in the mouse and how that might relate to fetal development in the human being.

7. SPECIAL EFFECTS

The liver appears to be a rather specific target for the biochemical effects of hexachlorobenzene.

When adult male rats were fed a diet containing 0.2% hexachlorobenzene, light and election microscopic studies revealed a marked enlargement of hepatocytes, many of which contained one or more discrete eosinophilic laminated cytoplasmic bodies. Proliferation of smooth endoplasmic reticulum, formation of large lipid droplets and protusion of hepatic cytoplasm through the space of Disse into the sinusoids were also observed (Medline, 1973).

At the biochemical level, short term feeding of HCB (0.2% in diet for 7 days) causes a marked increase in hepatic cytochrome P450 levels, enh ancement of the activities of several in vitro microsomal oxidations. The concentration of cytochrome P450 in the liver remains elevated throughout the chronic feeding of HCB (Stonard, 1974 , Turner, 1974, Grant, 1974) Since HCB has been shown to be associated with the induction of porphyria in human beings and experimental animals (Ockner, 1961, De Matteis, 1961) it has been suggested that the increase in the content of hepatic cytochrome P450 could be due to a direct effect of HCB on δ -aminoloevulinate synthetase, the first and rate limiting enzyme of the haem - biosynthetic pathway. In rats fed HCB (0.2% in diet) it has indeed been shown, that prolonged fungicide administration (2 - 3 weeks) is accompanied by a two-fold increase in δ-aminolaevulinate synthetase activity. The increase in enzyme activity however appears to be due to a decreased degradation rate of the enzyme rather than a stimulation of its activity. (Rajamanickan, 1972).

In another experiment, it was reported that feeding rats for 6 months a diet containing 0.2% HCB showed 50% mortality for the female and 20% for the male. Porphyrinuria was apparent at 10 - 12 weeks of poisoning. The simultaneous s.c. administration of 1 mg testosterone phenyl prqpionate twice weekly depressed in females the mortality to 10% and lengthened the initial porphyrinuria - free period to 15 weeks. In males, the simul-

taneous s.c. administration of 1 mg estrone acetate /day increased the mortality to 80% and shortened the porphyrinuria - free period to 7-8 weeks. (Niklos, 1973).

III BIOLOGICAL DATA ON HUMAN

Episodes of HCB toxicosis in man, associated with the consumption of HCB treated wheat, have been reported in Turkey (Cam, 1963, Schmid, 1960) Several hundred people, mostly children, were affected by the intoxication, which was diagnosed as porphyria-cutanea tarda. The syndrome consists of slistering and epidermolysis of the skin, involving exposed parts of the body, causing the skin to be unusually sensitive to sunlight and to minor trauma. The cutaneous lesions heal poorly and become easily infected. If healing does occur, pigmented scars form and contractures of the skin develop in regions where there is tissue loss. Moreover, infected skin lesions may give rise to suppurative arthritis and osteomyelitis affecting particularly the digits. There is usually marked hypertrichosis, which is not restricted to the exposed parts of the skin but covers the trunk and extremities with a fine layer of dark hair. The urine contained large quantities of porphyrins Weight loss and hepatomegaly were frequently present. Neurological symptoms did not appear to be evident but abdominal pain was reported in some cases and liver enlargment was present in 35% of the cases surveyed. In many cases bone and joint changes occurred; in some patients there was osteoporosis of the bones of the extremities and interphalangeal arthritis. It was estimated that the amount of HCB ingested by the persons affected was from 50 to 200 mg/day for a relatively long period before the disease became apparent.

After stopping the consumption of bread made from HCB - treated wheat the acute skin manifestations disappeared in about 20 to 30 days. Urinary findings reverted to normal in most patients. Relapses during the summer months were often seen. However one to two years after the termination of other symptoms of porphyria, the joint lesions were found to be still present. It was concluded that the disease was the result of a metabolic disorder caused by interference with porphyric metabolism in the liver by HCB. (Dogramaci, 1961, 1962, a, b, c, 1964, Wray, 1962).

Some other cases of porphyria have been reported in workers occupationally exposed to HCB (Morley, 1973).

In Australia (Brady, 1972), HCB has been reported in human body fat from trace amounts to concentrations as high as 8.2 ppm. In the same population (Sigali, 1972) and in a specimen of Louisiana population (Burns, 1975) HCB was detected in whole blood of more than 95% of the individuals studied. The average concentration was $\pm$ 4 ppb. Sign of apparent toxicosis were not observed in those studies except for a possible correlation between HCB residues and coproporphyrin and lactic dehydrogenase.

HCB blood residues were also found in 19 of 20 vegetable spraymen exposed to HCB contaminated dimethyl 2, 2, 3, 5, 6 tetrachloroterephtalate (DCPA). The mean level was 40 $\pm$ 63 ppb with a range of 0 to 310 ppb. No definite physical or biochemical effects of this exposure were discovered. Specifically there was no evidence of cutaneous porphyria or abnormalities of uroporphyrin or coproporphyrin excretion. A possible correlation however was found ($P < 0.05$) for HCB levels and aminolevulinate excretion (Burns, 1974).

As already mentioned earlier, hexachlorobenzene is found in human milk in concentration in the order of 1 mg/kg of fat (Luquet, 1972, 1975, Siyali, 1973, Miller, 1973).

At the european colloquium on "Problems raised by the contamination of man and his environment by persistant pesticides and organo-halogenated compounds" held in Luxembourg, May 14 - 15 1974, it was reported that in Germany, United Kingdom and Luxembourg the HCB concentration in human fat averaged respectively 4.8, 0.05 and 1.4 mg/kg fat respectively. In human milk in Luxembourg in 1974 the HCB content averaged 0.56 mg/kg fat.

IV RESIDUES IN FOOD

It is becoming more and more evident that the number of reports of residues of HCB in foods, feeds and human tissues increase. The sources of these residues are known to include disposal of industrial and municipal wastes, contamination of other chlorinated pesticides, the approval use of HCB as a seed-dressing, and misuse of HCB - treated seeds in animal feeds (WHO, 1975).

In addition, considerable data have been published indicating that HCB is a widespread contaminant of many food commodities, most particularly those of animal origin.

The Netherlands authorities in the course of inspecting imported animal feedstuffs found that certain milling fractions of raw cereals contained HCB residues ranging up to 3 ppm.

Raw cereals and their milling fractions are not the only source of HCB residues which find their way into animal feeds. Import inspection in the Netherlands has revealed consistent and relatively high levels of HCB in bran, beans, clover seed, chicory root, copra press cake, cotton press cake, lespedasia meal, maize meal, peas, potatoes, soybean meal and wheat middlings. Rice was one of the few commodities examined which was consistently free of HCB. Although the level of HCB residues found in imported feed commodities entering the Netherlands was lower than that of DDT residues, the frequency with which HCB residues occurred was much higher.

It was reported to FAO by the Swiss Federal Health Service that residues of HCB had been detected in various commodities as follows: (WHO, 1974)

meat fat	0.01 - 0.075 ppm
poultry fat	0.001 - 0.5 ppm
eggs (whoh)	0.002 - 0.3 ppm
milk fat	0.002 - 0.04 ppm
milk products	0.04 - 0.5 ppm
raw cereals	0.001 - 0.05 ppm

It has been reported in the Netherlands that broiler chickens had the tendency to accumulate HCB from contaminants in their feed. The results of this experiment indicate a direct relationship between the amount of HCB in the feed and the amount in the fat of the chickens (De Vos, 1972).

In hens that had been continuously fed HCB-treated wheat the body fat had HCB concentrations as high as 210 ppm and eggs from these birds contained as much as 70 ppm HCB (Brady, 1972). Concentrations of HCB in body fat greater than 300 ppm were reported in chickens fed HCB treated wheat (FAO, 1970).

It has been also reported that sheep store HCB in their body fat to the extent of 7 to 9 times the concentration in the feed at all gradation of intake from 0.1 to 100 ppm. The approximate half life of HCB in those fat varied between 10 and 18 weeks depending on the dose. (Avrahami, 1972).

When applied to soil, HCB has a half life of 969 - 2089 days (Beck, 1974). Since HCB is a common contaminant to quintozene, it has been show, that soil types from fields which had previously been pretreated with quintozene contained HCB residues at levels ranging from 0.17 to 0.94 mg/kg with an average of 0.38 mg/kg. Soil from fields which had not been treated for two or more years showed only slightly less HCB than soil from fields which had been treated more recently. Other studies have shown that such residues may be taken up by potatoes and carrots (Beck, 1973) or lettuce (Dejonckheere, 1974).

V CONCLUSIONS

Although the increase in the agricultural use of HCB is believed to be responsible for HCB residues in tissues of food-producing animals, it is becoming more and more evident that agricultural use of HCB is less important than industrial contamination of the environment as a source of exposure for animals. (Booth, 1975).

HCB has a low order of toxicity when animals are exposed on a short term basis. However, if the exposure to HCB is of long standing, the possibility of observing an adverse effect is much greater even at much lower level of exposure. Inasmuch as the biotransformation characteristics of HCB are unknown and the depletion from body tissues is slow, long-term exposure must be considered hazardous. Moreover, considerable biomagnification of HCB in the body fat of animals occur over an above the amount consumed in the diet during long-term exposure.

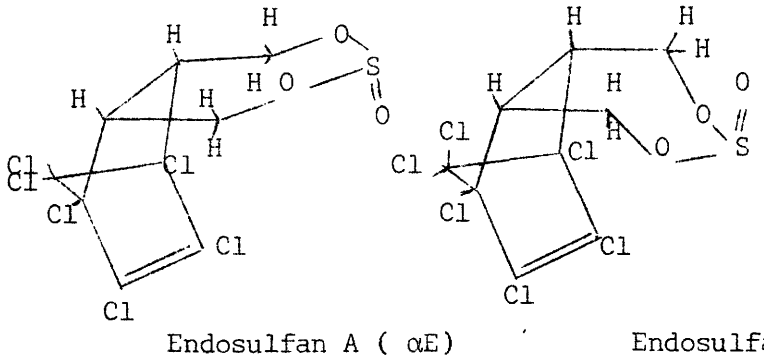
Although the literature contains good information on the toxicity of HCB, pharmacokinetoes studies, including histopathologic analysis, need to be conducted for all species of food producing animals. The excretion of HCB in lactating animals needs to be evaluated in terms of the degree to which it may be a hazard to the uckling newborn. In addition the effect of HCB on reproductive functions needs to be determined.

Informations is also needed regarding the effect of HCB on the hepatic microsomal enzyme systems and the possibility of interactions with other drugs or chemicals. Moreover, studies are needed to determine the extent of biodegradability of HCB in the environment, as weel as its biotransformation after an animal has been exposed to the chemical. Nothing is known concerning the potential carcinogenic effect of low level - long lasting HCB exposure. One even does not know if HCB is mutagenic.

Due to a lack of toxicologic informations, FAO (FAO, 1975) made only a suggestion for a conditional ADI of 0.0006 mg/kg b.w. Since it is clearly demonstrated that most of HCB contaminations come from industrial products rather than from its agricultural use, severe limits for and severe control of HCB content in industrial products such as for example quintoze are urgently needed.

ENDOSULFAN

$C_9 H_6 O_3 Cl_6 S$ M. W : 406, 95



Endosulfan A (αE) Endosulfan B (βE)

6, 7, 8, 9, 10, 10 - hexachloro - 1, 5, 5a, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3 - benzo - dioxathiepin - 3 oxide

α , β - 1, 2, 3, 4, 7, 7,-hexachlorobicyclo -(2,2,1) - hepten - 2 - bioxymethylene - 5, 6 sulfite

Hexachloro - 1, 9, 10, 11, 12, 12 oxydo - 5 - dioxa - 4, 6, thia - 5 tricyclo (7.2, $1.0^2.8$ dodecene - 10

Thiodan

Chlorthiepin

Insecticide: it is a contact and stomach insecticide specially active against various species of coleoptera and carterpillars. It is slightly toxic for the bees. It is in general non phytotoxic.

It is used as an insecticide before the crop and applied on fruit trees, vegetables, cereals, cotton, tobacco and tea.

I PHYSICO CHEMICAL PROPERTIES

The technical endosulfan contains two stereoisomers (WHO, 204) endosulfan A (α) (M.P., 106) and (WHO 205) endosulfan B (β) (M.P. 212) in the proportion variously reported as from 4:1 to 7:3

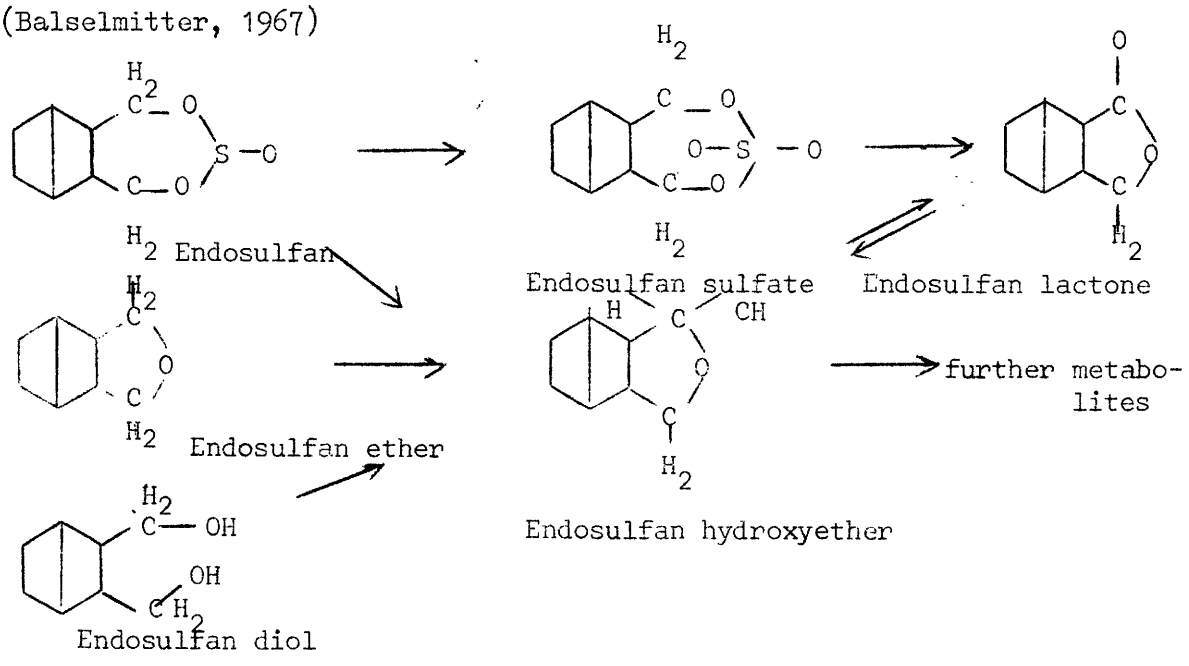
The technical material is a 90 - 95% pure mixture of the two isomers. It is a brownish crystallic solid which melts between 80 and 90°C. It is practically insoluble in water at 20°C and soluble in ethanol (5%) and in most of the organic solvents. It is only very slowly hydrolyzed in water, more quickly in acids or alcalis.

II BIOLOGICAL DATA IN LABORATORY ANIMALS

1. METABOLIC STUDIES

Undiluted endosulfan is only slowly and incompletely absorbed in the digestive tract of warm blooded animals. Absorption is more rapid in the presence of alcohols, oils and emulsifiers. These substances also accelerate the absorption of endosulfan through the skin (Maier-Bode, 1968).

Metabolic studies in rat and mouse, and using thin layer and gas-lipid chromatographic techniques have identified five of the possible metabolites of endosulfan: endosulfan sulfate (1), diol (5), ether (3), hydroxyether (4) and lactane (2) as illustrated in the following figure: (Balselmitter, 1967)



Endosulfan appears to be very rapidly metabolized and excreted in urine and faeces. Those compounds only slightly accumulated in the body.

Endosulfan given to mice in single doses or repeated daily for 49 days was found in the tissues as the sulfate (1). Oral doses of the isomers were partly excreted in the faeces unchanged along with endosulfan sulfate (1) and the diol (5). No residue was found in blood or brain, but traces of the sulfate (1) appeared in kidney and muscle (Deema, 1966).

When endosulfan was administered to rats, no unchanged endosulfan was found in the urine. Two metabolites were found in rat urine 48 hours after the animals were injected i.p. with endosulfan; these appeared to be conjugation products of its alcohol derivative (Rahn, 1963).

When endosulfan was fed to 3 female pigs at the rate of 2 ppm for up to 81 days, mean recoveries of endosulfan A and B and endosulfan sulfate (1) at 27, 54 and 81 days were 0.07, 0.09 and 0.04 ppm for body fat but none was found in other tissues and organs examined (Maier-Bode, 1968).

When endosulfan A or B were given p.o. or i.p. (4 or 8 mg/kg) to rats, the unchanged compounds, along with endosulfate (1), hydroxyendoether (4) and endolactone (2) were excreted in the faeces. Endodiol (5) and endoether (3) were not metabolites. In the urine after oral administration of the A or B isomers, endosulfan, endosulfate (1), endolactone (2) and an unidentified metabolite M1 were found. In the bile there were large amounts of endoketone and traces of M_2 after endosulfan A administration and large amounts of M_2 and traces of endolactone (2) after isomer B administration (Scherphan, 1968).

2. ACUTE TOXICITY

Animal	Products	Route	LD_{50} mg/kg b.w.	References
Rats	technical	p.o.	40-50 and 110*	FAO, 1968
			64 + 4	Truhaut
			18 to 43	Gaines, 1969
		i.p.	8	FAO, 1968
		skin	130 - 681	WHO, 1975
		inhalation	350 mg/m^3 (M)	WHO, 1975
			80 mg/m^3 (F)	WHO, 1975
	endo A	p.o.	76	Maier-Bode 1968
	endo B	p.o.	240	Maier-Bode 1968
Mouse	technical	p.o.	85 ± 5	Truhaut
	pure isomer	p.o.	15	Deema, 1966
Rabbits	technical	p.o.	147 - 359	WHO 1975
Hamster	technical	p.o.	118 ± 16	Truhaut
Dog	technical	p.o.	76	WHO, 1975

* Dependent on the vehicle used.

Truhaut further reported the oral doses of endosulfan which do not cause mortality as being 50 mg/kg för mouse, 40 mg/kg for rat and 70 mg/kg for hamster.

Fifteen days after such doses of endosulfan there are no significant biochemical effects except a decrease in the activity of hamster cholinesterase and an increase in the activities of rat and mouses. Serum LDH and GPT. Hamster liver glucose 6 phosphate dehydroginase activity is decreased and mouse liver GPT activity is increased.

From an histological point of view, lungs, heart, spleen, intestine testes and adrenals appeared perfectly normal while the liver was often congested and even in some cases in a degenerative process. In all cases it was clearly hypertrophied.

In short-term studies it was shown that rats tolerated 1.6 to 3.2 mg/kg b.w. orally for 12 weeks without any influence on growth rate (Czeck, 1958).

When endosulfan technical grade was administred daily for 3 days at a dose of 2.5 mg/kg b.w. to 4 dogs, vomiting was observed in one dog and vomiting, tremors, convulsions, rapid respiration and mydriasis in 3 dogs (FAO, 1968).

Endosulfan has been shown to be increasingly more toxic when given in large single oral doses to albino rats periously fed for 28 days from weaning on diets increasingly deficient in protein. The syndrome of intoxication was essentially the same in animals of all dietary group and consisted of stimulation of the central nervous system, an irritant gastro-enteritis and congestion of the brain and liver (Boyd, 1970).

3. CHRONIC TOXICITY

Three groups of dogs each consisting of 2 males and 2 females were given endosulfan orally in gelatin capsules 6 days a week for one year in doses corresponding to 0.25 and 0.75 mg/kg b.w. No signs of toxicity were observed. At autopsy gross and microscopic examination of the tissues showed no difference between treated and control animals (FAO, 1965 and 1968).

Groups of 25 males and 25 females rats received 10, 30ppm(0.5 and 1.5 mg/kg/day) endosulfan technical grade in the diet for 2 years with no histological signs of intoxication. At a dose of 100 ppm (5 mg/kg/day) during 2 years consistent histopathological findings were apparent both in liver and kidney (FAO, 1965, ,1968).

However, the survival of the female rats both in the 10 and 30 ppm groups was lower than that of the female control group during the second year. Moreover in the 100 ppm female groups, survival was significantly lower after 20 weeks and abnormalities were observed in weight gain and haematological examinatinn (FAO, 1965, 1968).

Groups of 8 males and 6 females rat were fed diets containing endosulfan 2 and 50 ppm (0.1 and 2.5 mg/kg/day) through three generations. No adverse effects were noted among either the parental animals or their progeny in all the generations. The growth, mortality, behavioural reactions organ weights, gross and histopathology, reproduction performances and survival indices of the progeny were comparable in both the test and control groups of animals (FAO, 1969).

4. CARCINOGENIC EFFECTS

No systematic analysis has been reported.

There is only one indication in a long-term study (2 years) that the TUMOR incidence was within normal limits in all test groups (FAO, 1965, 1968 and WHO, 1975).

5. MUTAGENIC EFFECTS

No reports available.

6. EMBRYOTOXIC EFFECTS

No reports available except a long-term study on rats which showed no adverse effects among either the parental animals or their progeny for up to 3 generations receiving diets containing 2 and 50 ppm of endosulfan (FAO, 1969).

7. SPECIAL EFFECTS

It has been reported that both endosulfan and endosulfan sulfate are highly toxic for fishes and many aquatic organisms (Canada/Ottawa, 1975). The insecticide is also toxic for some birds causing injurious action on their genital tracts, with as a consequence a reduced fertility of the males and females (Lutz, Ostertag, 1974).

III BIOLOGICAL DATA ON HUMANS

There are only very few informations concerning the effects of endosulfan on humans.

In U.S. 9 workers have had convulsions following endosulfan handling and 6 of them were known not to have had a history of previous convulsions while information on this point for the other 3 was not known. (Ely 1967).

In Asia, three workers have been intoxicated while handling endosulfan. Symptoms which appeared during the 24 hours following the contact were: agitation, headache and increaded irritability followed by vertiges, stupor and finally epilectic like convulsions (WHO, 1975).

Like for many other insecticides, it has been reported that endosulfan could persist on the hands of occupationally exposed workers for up to 112 days after exposure (Kazen, 1974).

Endosulfan has been reported as being one of the insecticides to which pesticide applicators with increased frequency of chromatid lesions were exposed (Yoder, 1973).

IV RESIDUES IN FOOD

Both endosulfan isomers are slowly hydrolysed to endosulfan alcohol and sulphur dioxide. Although in some way ressembling the cyclodiene structurally, the degree of persistence of endosulfan residues is not so great as for aldrin or endrine residues but is similar to that of lindane. Sunlight gives rise to some transformation to the most persistent

compound endosulfan sulfate, the toxicity of which is similar to that of endosulfan.

Field trials on blackcurrant have shown that unless deliberately oversprayed, the total residue of endosulfan A, endosulfan B and endosulfan sulfate does not normally exceed 0.5 ppm at harvest (FAO, 1968).

FAO and WHO have set tolerances for the sum of endosulfan A and B and endosulfan sulfate expressed as endosulfan. These tolerances for fruits and vegetables range from 0.1 to 2.0 ppm and there are no evidence reported so far showing that residues in the treated products were higher than these tolerances (FAO, 1975, n° 97).

One analysis of total food in one country has shown that only 1.2% of the agricultural products contained endosulfan. The average content was less than 0.005 ppm (WHO, 1975).

No endosulfan has been found in human fat (WHO, 1975).

V CONCLUSIONS

There is at least one report showing that endosulfan is one of the major chlorinated insecticides still in use today (Canada/Ottawa 1975). Since 1968 WHO/FAO have proposed an estimate of acceptable daily intake for man (for endosulfan A, endosulfan B and endosulfan sulfate) which they have fixed at 0 - 0,0075 mg/kg b.w.

It is evident however that the toxicological informations concerning this insecticide are very rare and even of it is rapidly metabolized and excreted and if it is not highly persistent its use calls for more studies.

Those studies might focus on its mutagenic, carcinogenic and teratogenic potencies which are, at the present time, completely unknown.

Due to its formation from endosulfan both chemically and biochemically, more work is required concerning the biological effects of endosulfan sulfate.

GENERAL CONCLUSIONS

As shown in the table which summarizes the toxicological data presently available concerning the 11 products reviewed the informations are, in most cases, only fragmentary. For most of the compounds it is not possible to propose ADI on a calculation basis and, except for Quintozenc and 2,4 D for which a complete set of informations exist, the reported values for maximum admissible daily intake are only "suggested" by WHO/FAO expert commitees.

For all the products, except Methoxychlor, Quintozenc and 2,4 D chronic toxicity studies have not been reported on other animals species but the rat. Acute toxicity has been measured in rodents only.

In most cases the metabolic pathways are not fully documented and many questions remain unanswered. Except for hexachlorbenzene (HCB) it seems that the reviewed pesticides do not accumulate in the body and that they are rapidly excreted. The report showing that repeated low doses of HCB (0,025 mg/Kg/day) could induce toxic effects raise an important question concerning the metabolism, tissue storage and excretion in animals and man chronically exposed to even very low level of those compounds. It must be noted also that in many cases the residues in fat, milk and food were reported to increases progressively : moreover for HCB the main source of contamination could be from industrial rather than agricultural use. It is also well known that 2,4,5 T are contaminated by 2,3,7,8 tetrachlorodibenzodioxine.

Concerning the embryotoxicity 2,4 D, 2,4,5 T, Quintozenc, PCP and HCB have been more or less carefully tested. For all the other products the litterature contains very few if any information. 2,4 D and 2,4,5 T both give rise to teratological effects, but the question still remains open concerning the possible role of TCDD which is the most potent teratogen we know. Pentachlorophenol is embryotoxic and the no effect level has been fixed at 5 mg/Kg/day. Hexachlorbenzene has been reported to induce some malformations but the data are not clear enough to draw final conclusions. Quitozenc does not seem to be teratogen.

The litterature available to the reporter is extremely poor concerning the carcinogenic and mutagenic potencies of the reviewed pesticides. There is no systematic multispecies analysis and in a few cases only mice have been used. None of the, in vitro mutagenesis tests, has been applied.

Except for HCB due to a dramatic story of population accidental exposure, the effects on man are poorly documented and it is very difficult to extrapolate from animal to man.

Due to the lack of information the suggestions of the reporter are the following :

- to carefully objectively and urgently reevaluate the use fulness of each product as compound with all the other available better known pesticides or fongicides;

- to severely define criteria of purity and "no contamination" rules for the useful products;

- to make strict recommandation to chemical industries in order to avoid environmental pollution with compounds as for example HCB;

- to define a program of control of the residues in food, fat, milk (including human) and environment;

- to make a choice of useful products for which toxicological, carcinogenic mutagenic and biotoxicological studies must be completed urgently.

Before such a program could be completed, it is suggested to apply extremely severe criteria and maximum ADI as low as 0,001 mg/Kg/bw for all the products except perhaps methoxychlor for which however more work is required.

For all the products, long term studies with low level exposure are required and the effects of low protein diet in their toxicity must be analyzed.

SUMMARY OF THE TOXICOLOGICAL DATA

	No effect level RAT		SAFETY FACTOR RAT	No effect level DOG		SAFETY FACTOR DOG	MAXIMUM A.D.I. mg/Kg/b.w.	ORAL LD_{50} mg/Kg				Estimated lethal dose for MAN mg/Kg
	ppm	mg/Kg		ppm	mg/Kg			MOUSE	RAT	DOG	RABBIT	
P.C.P.									27			
Quintozenc	25	2.5	2500	30	0.75	750	0.001		1700	2500	800	
2,4-D	500	50	1666	500	12,5		0,3	370	365-666	100	800	50-500
2,4,5-T	-	-		-	-		-	390	500	-	-	500-1000
MCPA	50	5		-	-		0,002	550	800	-	-	50
MCPB	-	-		-	-		-	-	680	-	-	-
Mecoprop	50	5		-	-		-	600	1060	-	-	-
Dichlorprop	-	12,4		-	-		-	400	800	-	-	-
Methoxychlor	200	20	200	-	300	3000	0,10	1850	5-7000	-	-	± 6.000
HCB	-	<0.025		-	-		0,0006	4000	3500	-	2600	-
Endosulfan	-	-		-	-		0,0075	15	40-60	76	147-360	-

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