The carbinole acaricides: Chlorobenzilate and chloropropylate

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I. Introduction

The two carbinole compounds chlorobenzilate and chloropropylate were synthesized by F. Häfliger in the Research Laboratories of *J. R. Geigy S. A.*, Basle, Switzerland.

Both chemicals displayed an excellent acaricidal activity in the biological tests carried out in 1951/52. Consequently, chlorobenzilate was introduced as a specific acaricide in 1952. Chloropropylate was not developed further, after the preliminary tests, since it showed no advantages over chlorobenzilate. It was not until 10 to 12 years later, when an increasing number of *Panonychus ulmi* and *Tetranychus urticae* strains in fruit growing had developed resistance to organic phosphates and satisfactory mite control with these chemicals was no longer warranted, that the acaricidal action of chloropropylate was studied anew and found to be promising in the control of resistant mites (Mathys 1963). The product is effective against most of the susceptible and resistant mite genera of *Tetranychidae* and *Eriophyidae* in orchards, vineyards, citrus plantations, and field crops, as well as in ornamental plants.

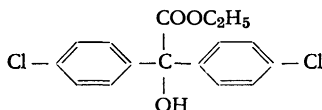
The objective of this review is to present all the pertinent published and unpublished data on chlorobenzilate and chloropropylate that have become available since their discovery and introduction as specific acaricides. The two compounds are dealt with separately in this review, because they were developed at different times and the respective experiments were therefore not conducted in parallel.

II. Properties and characteristics of chlorobenzilate

Chemical names: ethyl 4,4′-dichlorobenzilate
ethyl 2-hydroxy-2,2-di-(*p*-chlorophenyl)-acetate (IUPAC)

Empirical formula: $C_{16}H_{14}Cl_2O_3$

Structural formula:



Molecular weight: 325.18

Physical properties:

The pure compound is a yellowish, viscous liquid having a boiling point of 141° to 142° C. at 0.06 mm. of Hg. The vapour pressure is 2.2×10^{-6} mm. of Hg at 20° C., and 1.4×10^{-4} mm. of Hg at 60° C. The technical product contains approximately 93 percent of the above compound, is a brownish liquid with a specific gravity of 1.2816 at 20°/4°, and weighs 10.7 pounds/gallon. Chlorobenzilate is virtually insoluble in water, but is infinitely miscible in benzene, acetone, methyl alcohol, xylene, and deodorized kerosene.

Under normal storage conditions chlorobenzilate is stable whether stored as the technical material, or as a wettable powder, or an emulsifiable concentrate.

III. Toxicology of chlorobenzilate

a) Acute toxicity

The results of acute oral, dermal, and inhalation toxicity studies are shown in Table I.

b) Subchronic and chronic toxicity

1. Short-term studies (oral administration). —

α) *Rat.* — For 15 weeks, groups of five male rats were fed diets containing 0.05, 0.1, and 0.5 percent of technical chlorobenzilate (equivalent to 500, 1,000, and 5,000 p.p.m., respectively). All the animals of the 500 p.p.m. groups survived. In the 1,000 p.p.m. group two mortalities occurred, and in the 5,000 p.p.m. group all animals died within eight weeks (*Hazleton Laboratories* 1953 a).

In a 17-week study, rats were given 0.005 and 0.05 percent technical chlorobenzilate (equivalent to 50 and 500 p.p.m., respectively). The first group (50 p.p.m.) consisted of 20 males, the second group (500 p.p.m.) of 40 males and 20 females. Twenty males of the second group were sacrificed after 17 weeks of feeding for chemical analysis, and tissues from seven representative animals were histologically examined. The other groups remained under test as part of a two-year chronic feeding study which will be described below. Liver, spleen, lungs, thyroid, stomach, brain, testes,

Table I. *Acute oral, dermal, and inhalation toxicities of chlorobenzilate*

Animal	Route	Formulation or solvent	LD_{50} (mg./kg. body-weight) [a, b]	References
Mouse	oral	suspension in gum arabic	4,850	R. GASSER (1952)
Mouse	oral	25% wettable powder	3,200	*J. R. Geigy S. A.* (1954)
Mouse	oral	technical	729	H. J. HORN *et al.* (1955)
Rat	oral	suspension in gum arabic	3,100	R. GASSER (1952)
Rat	oral	technical	702	H. J. HORN *et al.* (1955)
Rat	oral	25% xylene emulsion	735	H. J. HORN *et al.* (1955)
Rat	oral		M 1,040; F 1,220	W. F. DURHAM (1967)
Rabbit	dermal	4 E (45.5% emulsifiable sol.)	> 5,000	*Ind. Bio-Test Lab.* (1965 b)
Rabbit	dermal	25% wettable powder	> 2,550	*Ind. Bio-Test Lab.* (1965 a)
Rat	aerosol inhalation	4 E; undiluted	LC_{50}[c] < 49 mg./l. air	*Ind. Bio-Test Lab.* (1965 b)
Rat	aerosol inhalation	4 E; 0.2% aq. sol.	LC_{50}[c] > 21 mg./l. air	*Ind. Bio-Test Lab.* (1965 b)
Rabbit	eye irritation test	25 W; 50 mg. of undiluted test material instilled into the conjunctival sac	moderately irritating	*Ind. Bio-Test Lab.* (1965 a)
Rabbit	eye irritation test	4 E; 0.1 ml. of undiluted test material instilled into the conjunctival sac	severely irritating	*Ind. Bio-Test Lab.* (1965 b)

[a] All values are expressed in terms of active ingredient.
[b] The symbol > means that the LD_{50} value is higher than the quoted figure, *i. e.*, the highest tested.
[c] The LC_{50} values are expressed in terms of the formulation used based on a four-hour exposure.

kidney, heart, large and small intestine, and adrenals showed no pathological changes which could be attributed to the administration of chlorobenzilate. Muscular tissue, fat, and brain were found to contain 3.9, 4.8, and 5.9 p.p.m. of chlorobenzilate, and in the liver 25.1 p.p.m. were found. Thus the amount stored was negligible in view of the daily dose of 500 p.p.m. (*Hazleton Laboratories* 1953 a and b).

In a 99-day feeding study, groups of 40 rats each (20 males and 20 females) were given a 20 percent chlorobenzilate powder which was added to the diet at dosage levels of 20, 100, 500, and 2,500 p.p.m., expressed in terms of active ingredient. From the criteria chosen for the evaluation of the biological activity it was concluded that the intake of 20, 100, and 500 p.p.m. of chlorobenzilate did not cause any noticeable toxic effect in the treated animals. All 120 animals in these three test groups behaved the same as the controls, also in relation to autopsy and histological findings. The dosage level of 2,500 p.p.m. caused a significant retardation of the weight gain from the third or fourth week onwards, and upon completion of the test the overall weight gain was 91 percent of the initial weight as compared to 147 and 143 percent in the control groups. Upon inspection, the general appearance and behaviour of the animals was normal. During the whole test, only one male out of 40 animals in the 2,500 p.p.m. group died on the 17th day. Autopsy revealed no gross pathological changes, and the organ weights showed no deviation from controls. The histological examination of selected tissues revealed a noticeable increase of fatty deposit in the liver lobules of three rats. However, there were no degenerative changes of the epithelium nuclei or of the cytoplasm. The centrolobular glycogen content was appreciably reduced, but there was no evidence of degenerative liver damage. Five out of the 20 males in this series exhibited signs of spermiogenetic injury and testicular atrophy (DOMENJOZ 1965 a).

In view of the fact that dichlorobenzilic acid is a metabolite in the degradation of chlorobenzilate in warm-blooded animals, a 99-day feeding study with dichlorobenzilic acid in rats was conducted in parallel to the 99-day test with chlorobenzilate. The test substance was added to the diet at dosage levels of 20, 100, 500, and 2,500 p.p.m. All the animals in these test groups (four series of a total of 160 rats) behaved exactly as the two control groups (untreated group and a placebo formula 2,500 p.p.m. group) in respect of the criteria chosen for the evaluation of a possible toxic effect of the material (general condition, food consumption, body-weight gain, mortality, autopsy, weight of organs, and histological findings). It is, therefore, concluded that 2,500 p.p.m. of dichlorobenzilic acid is a no-effect level (DOMENJOZ 1965 b).

Groups of 20 male rats each received technical chlorobenzilate in their diet at levels of 0.004 percent (40 p.p.m.) and 0.08 percent (800 p.p.m.) for 48 and 44 weeks, respectively. In the 0.004 percent group rats exhibited normal growth and food consumption during the 48 weeks. No deaths occurred in the animals at this level. Mortality was slightly higher in the 0.08 percent group than in the control group. The rats of the 0.08 percent

group exhibited growth retardation throughout the 44 weeks of feeding, and several animals showed red or swollen eyelids and soft feces. Organ-to-body-weight ratios revealed that the livers, kidneys, and testes of the rats of the 0.004 percent group were significantly heavier than the respective organs in the control rats. In the 0.08 percent group liver and kidney weights of the rats appeared to be heavier than those of the controls, but were not statistically evaluated. Upon autopsy no gross pathological changes were observed which could be attributed to the administration of the compound, but the histological examination revealed increased hemopoietic activity of the spleens in the 0.08 percent group, and non-specific changes in the pancreas and adrenal organs of the rats in both test groups.

Tissue analyses of liver, kidney, fat, brain, and muscles showed that chlorobenzilate is not stored in the animal body to any appreciable extent (*Hazleton Laboratories* 1953 c).

β) *Dog.* — Technical chlorobenzilate was administered orally by capsule five days a week for 35 weeks at dosage levels of 12.8 and 64.1 mg./kg./day to groups of two dogs, each group consisting of one male and one female. All dogs either maintained their body-weight or made slight weight gains; they exhibited normal behaviour, had good appetite, and showed no gross signs of toxicity throughout the entire study. Biochemical and hematological findings were within normal limits. Gross and microscopic examination revealed no significant pathological changes at the end of the 35-week period which could be attributed to the oral ingestion of chlorobenzilate (HORN *et al.* 1955).

γ) *Sheep and beef-cattle.* — In a sheep and cattle study, Chlorobenzilate 25 W was fed with the grain to eight sheep and six beef cows over a period of four weeks, according to the following scheme:

Two sheep	(females)	0
Two beef-cattle	(male and female)	0
Two sheep	(females)	3.6 p.p.m. active ingredient
Two sheep	(females)	8.7 p.p.m. active ingredient
Four sheep	(3 females, 1 male)	29.0 p.p.m. active ingredient
Two beef-cattle	(male and female)	3.2 p.p.m. active ingredient
Two beef-cattle	(male and female)	8.8 p.p.m. active ingredient
Two beef-cattle	(male and female)	27.0 p.p.m. active ingredient

Based on the criteria chosen for the evaluation, viz. food consumption and bowel evacuations, physical condition and behaviour, hematology and gross necropsy observations, no compound-related toxic effects were noticed. One cow dosed at 3.2 p.p.m. aborted a six-month fetus on the fourth day. However, a second cow at 8.8 p.p.m. level showed a fully developed fetus in the uterus upon the completion of the study (*Woodard Research Corp.* 1965 a).

2. Long-term studies (oral administration). —

α) *Rat.* — In the two-year chronic feeding study, part of which was described above, there was no significant difference in mortality or food

consumption between the treated groups and their controls, but the males of the 500 p.p.m. group had a significantly lower weight gain than the controls. Signs of toxicity, consisting of a blood-tinged crust over the nose and eyes, and unthriftiness, were noted in both males and females at 500 p.p.m. In the males receiving 500 p.p.m. of technical chlorobenzilate there was a greater incidence of atrophic testes, but the histological picture was not different from the controls. Other signs of gross and microscopic pathology were observed as frequently in the control groups as in the experimental groups (HORN *et al.* 1955).

In a further two-year chronic feeding study, Chlorobenzilate 25W was administered in the diet to four groups of 60 rats each (30 males and 30 females) at levels corresponding to zero, 40, 125, and 400 p.p.m. of chlorobenzilate active ingredient. Criteria chosen for the evaluation of the biological activity were general appearance, behaviour, survival, body-weights, food consumption, clinical chemistry, gross pathology, organ weights, and histological pathology including frequency of neoplasms. As judged by these criteria there was no adverse effect noted in the rats of the 40 p.p.m. group. In the 125 p.p.m. group, the gross pathological and histopathological examination gave some evidence of more frequent testicular changes. In the 400 p.p.m. group, the following effects were noticed: moderate increase of mean relative liver weight of males and slight increase of mean absolute liver weight of females, more frequent hepatic and renal changes, as well as more frequent testicular changes (*Woodard Research Corp.* 1966 b).

β) *Dog.* — In a two-year study, four groups of dogs consisting of three males and three females each received Chlorobenzilate 25W in the diet at the following dosage levels (expressed in terms of active ingredient): zero, 100, 500, and 5,000 p.p.m. At the end of the 14th week two male and two female dogs of group no. 4 (5,000 p.p.m.) were selected at random, removed from compound, and placed on control diet for five weeks. At the end of five weeks the four dogs were replaced on chlorobenzilate at the reduced dietary level of 3,000 p.p.m. One male and one female dog from group no. 4 continued on the 5,000 p.p.m. dietary level through 20 weeks and then were sacrificed. Based on the parameters considered (appearance, body weight gains, appetite, survival, hematological and biochemical studies, organ weights and organ/body weight ratios, gross and histological pathology) 100 and 500 p.p.m. of chlorobenzilate produced no effect. The administration of 5,000 p.p.m. of chlorobenzilate caused, within 13 to 14 weeks, poor appetite and weight loss, moderately severe anemia, increase of serum alkaline phosphatase, lowering of serum protein, and reversal of the albumin/globulin ratio. Withdrawal of the high dose level (5,000 p.p.m.) from the diet of four dogs for five weeks resulted in clinical improvement, with recovery of appetite and weight loss and essentially a recovery from the anemia, with return to normal of the alkaline phosphatase and serum protein values. A dietary level of 3,000 p.p.m. administered for 80 weeks, following an initial feeding of 5,000 p.p.m., was found to be a minimal effect level. The dogs essentially maintained normal appearance and be-

haviour, but showed a tendency toward a mild anemia. Gross pathological examinations and organ weight data indicated enlarged livers and spleens for several of the dogs of the 5,000 p.p.m. group. Histological examination revealed extramedullary hematopoiesis in liver and spleen, and erythroid hyperplasia of the bone marrow was evident particularly in the dogs of the 5,000 p.p.m. group that were sacrificed after 20 weeks; it was also present among the dogs that received the reduced level of 3,000 p.p.m. and were sacrificed after two years (*Hazleton Laboratories* 1965 a).

3. Repeated dermal application. —

α) *Rabbit.* — A 21-day subacute dermal toxicity study (15 applications) was conducted with Chlorobenzilate 25W at dose levels of 1.0 and 2.0 g./kg. per day. The results of this study did not reveal any significant adverse findings in respect of the parameters chosen for the evaluation of a possible effect such as behavioural reactions, body-weight, mortality, hematology, urine analysis, clinical chemistry, organ weight, and gross and histological pathology. Local skin reactions, characterized by erythema, dryness, and cracking of the skin at the application site, were noted among all test animals during the test period. Desquamation occurred at the end of the experiment, but only a slight thickening of the skin was noted (*Industrial Bio-Test Laboratories* 1965 c).

A similar 21-day subacute dermal toxicity study (15 applications) was conducted with Chlorobenzilate 4E (45.5 percent chlorobenzilate emulsifiable solution). Skin applications of the undiluted test material were made to four groups consisting of 10 rabbits of both sexes (intact and scarified skin) at dose levels of 1.0 and 2.0 g./kg./day (corresponding to 456 and 912 mg./kg./day of chlorobenzilate active ingredient). The results were as follows: One male in the 1,000 mg./kg./day group (scarified skin) and two females in the 2,000 mg./kg./day group (scarified skin) died. Death of the male and one female was due to a respiratory infection while the immediate cause of death of the other female was not determined. No other deaths occurred among the animals in the four groups. Local skin reactions, characterized by moderate to severe erythema, severe edema, cracking, subdermal hemorrhaging, and surface bleeding were observed in the animals of the four test groups. The body-weight of the animals was depressed in all the four test groups. With the exception of the skin at the application site of the animals in the 2,000 mg./kg./day group that died, no significant gross pathologic and histologic changes were observed that could be attributed to the heavy exposure of repeated applications of an undiluted 45.5 percent chlorobenzilate emulsifiable solution (*Industrial Bio-Test Laboratories* 1967 a).

β) *Man.* — A repeated insult patch test was conducted in 56 human subjects on Chlorobenzilate 4E (two percent aqueous emulsion) and on technical chlorobenzilate (one percent aqueous emulsion). Under the conditions of this test neither material produced significant primary irritation and no evidence of sensitization was obtained. Both materials tended to produce

mild skin fatigue effects in some subjects, with Chlorobenzilate 4E being slightly more active than technical chlorobenzilate in this respect (*Hill Top Research Institute* 1963).

c) Special studies

1. Reproduction in the rat. — A three-generation reproduction study was carried out on rats receiving 50 and 25 p.p.m., respectively, of chlorobenzilate active ingredient in the diet over a total period of 88 weeks. The compound was fed as a 25 percent wettable powder. Based on the criteria used (e. g., biweekly body weights, observations at birth and at weaning of litters from each of two matings/generation, various organ and uterine implantation site examinations), it was concluded that the reproductive performance of rats given the above-mentioned doses of chlorobenzilate in the diet was essentially comparable to that of the control rats. No teratogenic changes were observed in any of the young born and all offspring appeared normal (*Woodard Research Corporation* 1965 b).

2. Effects on fish and wildlife. —

α) Wild birds. — Bobwhite quail (Colinus virginianus). Seven groups consisting of 10 bobwhite quails each were fed Chlorobenzilate 25W at dietary levels of 20,000, 10,000, 5,600, and zero p.p.m. (equivalent to 5,000, 2,500, and 1,400 p.p.m. of active ingredient) for seven days in comparison to 77.2 percent *p,p'*-DDT at levels of 560, 180, and 80 p.p.m. In terms of active ingredient the LC_{50} was found to be 3,375 p.p.m. of chlorobenzilate and 432 p.p.m. of *p,p'*-DDT. DDT was therefore 7.8 times more toxic than chlorobenzilate.

Mallard ducks (Aras platyrhynchus). Eighteen groups of 10 mallard ducks each were maintained for five days on diets containing 32,000, 18,000, and 10,000 p.p.m. of Chlorobenzilate 25W and of 320 to 10,000 p.p.m. of 77.2 percent *p,p'*-DDT. In terms of active ingredient the LC_{50} for chlorobenzilate was >8000 p.p.m., and that for *p,p'*-DDT 525 p.p.m. Chlorobenzilate is therefore considerably less toxic than DDT to mallard ducks.

β) Fish. — Rainbow trout (Salmo gairdneri). Groups of five fish each were placed in glass jars containing 15 liters of water and exposed for 96 hours to concentrations of chlorobenzilate ranging from 0.18 to 10.0 p.p.m of the active ingredient and to *p,p'*-DDT at concentrations of 0.0018 to 0.0056 p.p.m. active ingredient. Mortality was recorded during each 24-hour period. After 96 hours of exposure the LC_{50} was as follows: chlorobenzilate 0.60 p.p.m., and *p,p'*-DDT 0.0022 p.p.m. Chlorobenzilate was therefore 272 times less toxic than DDT.

Bluegill sunfish (Lepomis macrochirus) and goldfish (Carassius auratus). In similar fish toxicity tests as described above, the following LC_{50} values for these two species were determined based on a 96-hour exposure (Table II).

Chlorobenzilate is, therefore, markedly less toxic than DDT to these two species tested (*Woodard Research Corp.* 1965 c).

Table II. *LC_{50} values for two species of fish*

Species	LC_{50} (p. p. m. active ingredient)	
	Chlorobenzilate	*p,p'*-DDT
Bluegill sunfish	1.8	0.0034
Goldfish	0.71	0.0023

γ) Oyster (Ostrea virginica). — Oysters were exposed to Chlorobenzilate 25W and *p,p'*-DDT in flowing sea water aquaria for one week. Based on mortality and shell growth as criteria, it was found that Chlorobenzilate 25W in a concentration of one p.p.m. did not inhibit shell growth, whereas 0.24 p.p.m. of *p,p'*-DDT reduced shell growth by 50 percent. One p.p.m. of Chlorobenzilate 25W caused no mortality, while *p,p'*-DDT at 0.5 p.p.m. produced 35 percent mortality (*Woodard Research Corp.* 1966 a).

d) Acceptable daily intake

On the basis of the present toxicological data the 1968 Joint FAO/WHO Meeting of Pesticide Residues established as acceptable daily intake (ADI) for man a value of 0.02 mg./kg. body-weight [*World Health Organization*, Techn. Report Series No. 417 (1969)]. Therefore, a 60-kg. person would tolerate a daily dosage of 1.2 mg. of chlorobenzilate without any appreciable risk.

IV. Metabolism of chlorobenzilate in animals and residues in meat and milk

Metabolic studies following oral administration of chlorobenzilate have been performed in dogs, sheep, and cows.

In a dog study, chlorobenzilate was administered orally to four mongrel dogs five days a week for a total of 35 weeks at dosage levels of 12.8 and 64.1 mg./kg./day. Urine and feces were collected for a total of 10 days. During the second five-day period the compound was not administered, in order that the rapidity with which the compound was excreted could be followed. The analytical results showed that chlorobenzilate was excreted in the urine in large quantities, an indication that the compound was absorbed rapidly and excreted through the kidneys. At 12.8 mg./kg./day, the male excreted 42.67 percent of the total dose in the urine and the female 41.84 percent. At 64.1 mg./kg./day the corresponding values were 21.77 and 15.13 percent, respectively. Only small amounts of chlorobenzilate were found in the feces (4.30, 7.54, 6.61, and 6.60 percent, respectively). After the compound was withheld, the amount of chlorobenzilate could be analyzed following the third day of withdrawal from the compound. These studies were performed twice during the chronic feeding experiments with the dogs, with essentially identical results. At termination of the study, the dogs were sacrificed and their tissues analyzed (blood, liver, kidney, fat,

muscle, and brain). It was found that chlorobenzilate was not stored in any of the tissues examined (HORN *et al.* 1955).

In a similar dog study, chlorobenzilate was administered to three groups of mongrel dogs daily for five days at the same levels of zero, 12.8, and 64.1 mg./kg./day. Each group consisted of one male and one female. Daily urine and total fecal specimens pooled were analyzed. At 12.8 mg./kg./day, the male excreted 30.98 and the female 41.12 percent of the total dose in the urine within 10 days. In the feces, 5.58 and 6.64 percent, respectively, were found. At 64.1 mg./kg./day, urinary and fecal elimination was 31.20 and 1.78 percent, respectively, for the male, and 22.86 and 26.04 percent for the female. The results of this study are in good agreement with the data reported by HORN *et al.* (1955). At the end of 10 days, each animal, including the two controls, were sacrificed and the blood, brain, fat, liver, kidney, and muscle tissues were analyzed for residues. The analytical results showed that no stored chlorobenzilate was found in any tissues (*Hazleton Laboratories* 1964). Chlorobenzilate hydrolyzes at the ester linkage resulting in the formation of dichlorobenzilic acid. This compound is determined by the Schechter-Haller procedure. However, thin-layer chromatography (TLC) was used for qualitative identification purposes. Ether extract of urine from the treated dogs contained a material which, when chromatogrammed, corresponded to dichlorobenzilic acid (MATTSON *et al.* 1965). The results of the TLC analysis are shown in Table III.

Table III. *TLC analysis of urine from treated dogs*

Sample	Material administered	Days after treatment	Dichlorobenzilic acid
Control urine	—	—	absent
Standard dichlorobenzilic acid in control urine	—	—	present
Urine from dog given 64.1 mg. of chlorobenzilate/kg./day	chlorobenzilate	1	present
	chlorobenzilate	2	present
	chlorobenzilate	5	present
	chlorobenzilate	6	present

In a sheep and cattle study a daily ration of mixed timothy and clover hay supplemented with 16 percent protein mixed grain containing Chlorobenzilate 25W was fed to eight sheep and six beef cattle (both sexes) over a period of four weeks (*Woodard Research Corp.* 1965 a). The dosage levels were 10, 24, and 80 mg. of chlorobenzilate/animal/day for sheep (equivalent to 3.6, 8.7, and 29 p.p.m. of active ingredient) and 40, 110, and 340 mg. of chlorobenzilate/animal/day for cattle (equivalent to 3.2, 8.8, and 27 p.p.m. of active ingredient).

Tissue samples were analyzed for storage of chlorobenzilate by a colorimetric procedure (Schechter-Haller). Those samples found to contain measurable residues were also analyzed by microcoulometric gas chromatography. Agreement by the two methods demonstrated the reliability of the results and proved that unchanged chlorobenzilate was determined. The tissues analyzed were subcutaneous fat, omental fat, perirenal fat, longissimus dorsi, biceps femoris, liver, heart, kidney, and blood (MATTSON *et al.* 1966). Residues of unchanged chlorobenzilate were found only in the cattle and only at the highest feeding level of 340 mg./animal/day as shown in Table IV.

Table IV. *Residues in fat tissues of cattle*

Tissue	Residue (p. p. m.)	
	Males	Females
Subcutaneous fat	0.54	0.32
Omental fat	0.98	0.69
Perirenal fat	0.54	0.69

In another feeding study technical chlorobenzilate was administered to six dairy cows for a period of 15 days at dietary levels of 160, 48, and 20 p.p.m. in four pounds of feed. These levels of chlorobenzilate were based on the information that citrus pulp may be incorporated into commercial cattle feed at a level to provide for not more than 16 pounds of dried citrus pulp/cow/day. Assuming this citrus pulp to contain 40 p.p.m. of chlorobenzilate, and that 16 pounds might be fed/day/animal, the high level for this study became 160 p.p.m. in four pounds of feed. Milk samples were taken on the 2nd, 4th, 6th, 9th, and 15th days of the study. On the 16th day, chlorobenzilate feeding was terminated and the animals were given control feed supplement to permit post-treatment analysis of milk samples. Therefore, milk samples were again taken on the 20th, 21st, and 24th day of the study. Chlorobenzilate in the milk was found in one "high level" cow (160 p.p.m.) on the second day (0.15 p.p.m.) and in one "middle level" cow (48 p.p.m.) on the fourth day (0.15 p.p.m.), but in no case more than 0.06 p.p.m. on the 15th day. Since these values are approaching the limit of sensitivity of the method used, since they lack correlation with the feeding levels, and since a number of negative values were obtained, the few positive values may be entirely fortuitous. On the basis of the commonly accepted amount of hay eaten/day by a dairy cow (two pounds of hay/100 pounds body weight) and using an average weight of 1,000 pounds (actually 800- to 1,000-pound cows were used), the chlorobenzilate feeding levels used would correspond to 27, 8, and 3.3 p.p.m., respectively, in the total diet in this experiment. No free dichlorobenzilic acid was found in the milk. Some limited experiments indicated that free dichlorobenzilic acid, if present in the milk, would have been detected by the methods used (*Woodard Research Corp.* 1960).

V. Performance of chlorobenzilate

Already in the first biological tests in the laboratory and in the field chlorobenzilate proved to be a specifically selective acaricide (GASSER 1952). Today it is an appropriate product for the control of mites in fruit, vine, and vegetable growing as well as in cotton, tea, and particularly citrus plantations. The product has gained in significance mainly in recent years since it proved to be well suited for the control of multi-resistant mite strains.

a) Mode of action

1. Acaricidal action. — Chlorobenzilate is active against *summer eggs* and all *postembryonic stages* of most plant-injuring mites. In a laboratory experiment (GASSER 1952) the efficacy of various concentrations against eggs and mobile and dormant stages of *Tetranychus urticae* Koch was investigated (Table V). The mobile stages are the most harmful, but also the most susceptible ones. Already after a period of 24 hours, they are completely killed with 10 g. a.i./100 l. Also in the dormant stages and eggs the mortality reaches 100 percent six days after treatment. Chlorobenzilate is a *true ovicide;* when the active ingredient is sprayed on eggs, it penetrates the various egg layers (outer wax layer, cement layer, inner layer) and destroys the egg after 43 to 46 hours. The larvae that hatch from eggs laid after the treatment are killed by the remaining deposit of active ingredient (ovolarvicidal action) (HOPP 1954).

Chlorobenzilate is a *contact acaricide, i e.,* the best control is obtained when the mites come into direct contact with the active ingredient. Adults of *Tetranychus urticae* were placed on both surfaces of bean leaves one side of which had been sprayed with chlorobenzilate. With a dosage of 16 g. a.i./100 l., 50 percent of the mites present on the treated surface were killed; in order to obtain a 50 percent mortality on the untreated surface a tenfold dosage was required (EBELING and PENCE 1954). This experiment shows that the product hardly goes through the whole leaf, in other words that the product has no penetration effect.

Table V. *Concentration vs. efficacy against T. urticae Koch*

Concentration of chlorobenzilate (%)	Percentage of stages [a] destroyed after								
	1 day			3 days			6 days		
	M	D	E	M	D	E	M	D	E
0.1	100	0	0	100	37	32	100	100	100
0.05	100	0	0	100	20	16	100	100	100
0.01	100	0	0	100	25	23	100	100	98

[a] M = mobile stages (larvae, protonymphs, deutonymphs, adults).
D = dormant stages (nymphochrysalides, deutochrysalides, teleochrysalides).
E = eggs.

2. Duration of action. — The duration of action of a chlorobenzilate deposit tested in the laboratory is six days. During this period treated bean leaves (dosage 20 g. a.i./100 l.) cannot be artificially infested with *T. urticae*. After the sixth day the action of the deposit is reduced. The chlorobenzilate deposit on citrus crops remains effective for a longer period of time. Lemons and oranges treated at 30 g. a.i./100 l. were free of *Aceria sheldoni* for one to two months and of *Brevipalpus lewisi* for six to seven weeks (JEPPSON *et al.* 1957 a and b).

The activity of the product on cotton lasts six weeks.

3. Effect on predators and bees. — Owing to its specifically acaricidal action chlorobenzilate spares the useful insects (BARTLETT 1963). Insects (hyperparasitic Hymenoptera, predatory Coccinellidae) are not affected by chlorobenzilate treatments at all. The build-up of predatory mite populations of the genera *Amblyseius* Berl. and *Typhlodromus* Scheuten is only sligthly affected by chlorobenzilate treatments (MÜLLER 1960, SWIRSKI *et al.* 1967).

The selectively acaricidal action of chlorobenzilate mainly shows in the fact that it is being used for the control of the acarine disease of bees without affecting the honey bees themselves (GASSER 1966). The innocuity of chlorobenzilate to bees is often referred to in literature. According to ATKINS and ANDERSON (1954), chlorobenzilate belongs to the moderately toxic compounds (LD_{50} value for *Apis mellifera* L. is 16 mg. a.i. of a four percent dust formulation after 72 hours). Basing on laboratory experiments, BEYE *et al.* (1959) come to the conclusion that chlorobenzilate is not noxious to bees: bees were placed on a contact paper of 150 $cm.^2$ treated with eight mg. a.i.; no symptoms were observed after 24 hours. According to JOHANSEN (1966), who had investigated 92 insecticides and acaricides for bee toxicity, chlorobenzilate is moderately toxic to bees under laboratory conditions, but in the field (field conditions) it is only slightly toxic or safe to bees. Treatments with chlorobenzilate can, therefore, be carried out at any time.

b) Fields of application and recommendations

In the following Tables VI through X the mite genera are compiled according to the crops and the recommended dosages necessary for their control are presented. Chlorobenzilate treatments are generally indicated when the first mite damages on the crops occur. Since chlorobenzilate is a contact acaricide spraying must be very thorough, so that the entire surface of the plant is wetted. If infestation is very heavy, it may be necessary to carry out a second treatment seven to 10 days after the first.

1. Fruits. — Apples, pears, cherries, grapes, almonds, walnuts, and berries (Table VI).

At first, chlorobenzilate was used in fruit growing and mainly against the red spider mite *(Panonychus ulmi)*. In the Rhône Valley (Switzerland)

treatments with 25 g. a.i./100 l. reduced a *Panonychus* population on apples after 15 and 22 days, respectively, by 93 and 89 percent, respectively (GASSER and GROB 1963). For the control of *Tetranychidae* and *Eriophyidae* in fruit growing a dosage of 30 to 45 g. a.i./100 l. is recommended today, the lower dosage being applied for a repeated treatment after seven to 10 days. The higher dosage is indicated where mites that are resistant to organic phosphates or other acaricides must be controlled. For treatments of entire orchards of apples and pears 3 to 3.5 kg. a.i./ha. and of nuts and almonds 3.5 to 4.5 kg. a.i./ha. are necessary. Frequently lower dosages will

Table VI. *Mites and recommended dosages for control*

Mite		Recommended dosages		
		In U. S. units		In metric units
Genus and species	Common name	4 E [a] (pt./100 gal.)	25 WP (lb./100 gal.)	Active ingredient (g./100 l.)
Tetranychidae				
Panonychus ulmi Koch	European red mite	1/2—3/4	1—1 1/2	30—45
Tetranychus urticae Koch	two-spotted spider mite	1/2—3/4	1—1 1/2	30—45
Tetranychus pacificus McGregor	Pacific spider mite	1/2—3/4	1—1 1/2	30—45
Tetranychus mc danieli McGregor	McDaniel mite	1/2—3/4	1—1 1/2	30—45
Tetranychus schoenei McGregor	Schoene spider mite	3/4	1 1/2	45
Tetranychus cinnabarinus Boisduval	carmine mite	1/2	1	30
Bryobia praetiosa Koch	clover mite	1/2—3/4	1—1 1/2	30—45
Bryobia rubrioculus Scheuten	brown spider mite	1/2—3/4	1—1 1/2	30—45
Eotetranychus carpini vitis Oudemans	—	1/2	1	30
Eriophyidae				
Aculus cornutus Banks	peach silver mite	1/2	1	30
Aculus fockeui Nalepa	plum nursery mite	1/2	1	30
Aculus schlechtendali Nalepa	—	1/2	1	30
Phyllocoptes gracilis Nalepa	dryberry mite	1	2	60
Eriophyes vitis Pagst	grape erineum (bud) mite	1/2	1	30
Epitrimerus pyri Nalepa	pear rust mite	1/2	1	30

[a] 4 E = 45.5 percent of active ingredient.

do. DOWNING (1958) obtained a good effect against *Panonychus ulmi* and *Bryobia* sp. already with 2.2 kg. a.i./ha.; this, however, was at a time where the resistance described above had hardly occurred yet. The most favourable type of application of chlorobenzilate is the high-volume method, for only thorough wetting of all the infested plant parts warrants good results.

Chlorobenzilate is particularly suited for summer treatments when all the mite stages are present and when the useful insects must be spared (GASSER 1955). Summer foliar sprays at 37.5 to 45 g. a.i./100 l. were effective in reducing and maintaining the mite population *(P. ulmi)* at low levels for the remainder of the season (CLEVELAND 1958, OATMAN 1959).

The pear rust mite, *Epitrimerus pyri*, that mainly attacks the pear varieties Bartlett, Anjou, and Comic can be controlled satisfactorily with one summer treatment (30 g. a.i./100 l.) (WESTIGARD and BERRY 1964). Equally good results were obtained with 30 g. a.i./100 l. against *Aculus cornutus* on peaches and *A. fockeui* on cherries (ANTHON 1954 and 1957).

Phyllocoptes gracilis, an *Eriophyidae* which causes the young fruits of loganberries and raspberries to dry up can be well controlled with two chlorobenzilate treatments in March and July (BREAKEY and BATCHELOR 1957). In vineyards mite control must commence when the first damage (discoloration of leaves) becomes visible. Late treatments are also recommendable since oviposition in winter can be reduced by them and consequently also the damage on sprouts in the following spring.

2. Citrus. — Oranges, lemons, tangerines, and grapefruit (Table VII).

In citrus crops chlorobenzilate is well introduced. Already in the first field trials it was more efficient against the three major mite pests *Aceria sheldoni*, *Phyllocoptruta oleivora*, and *Brevipalpus lewisi* than the conventional petroleum oils sprays (JEPPSON 1955). A dosage of 30 g. a.i./100 l. or 2.75 kg. a.i./ha., respectively, is sufficient for the control of most mite genera. For the elimination of *A. sheldoni*, *Eotetranychus yumensis*, and *Tetranychus pacificus* 30 to 45 g. a.i./100 l. or 4 kg. a.i./ha., respectively, should be applied, whereas against rust mites 15 to 30 g. a.i./100 l. are generally sufficient. Also, in this case, the success depends on the thorough wetting of all the plant parts. The spray applications are carried out with 50 to 75 l. of water/tree or with 2,000 to 20,000 l./ha. (according to number and dimension of the trees) at high pressure (ELMER and JEPPSON 1957). In aerial applications (fixed-wing aircraft or rotory wing aircraft) only 93 l. spray volume/ha. are necessary. With such a treatment (dosage three pt. of chlorobenzilate 25 E in 10 gal./acre) a *Phyllocoptruta* attack could be satisfactorily kept down for two to three months (BULLOCK 1965).

Chlorobenzilate treatments are being carried out in summer as soon as the mites are visible. A repeated application may prove to be necessary in the case of a *Panonychus citri* attack. In order to sufficiently keep down an *Aceria* attack on lemons, a single application will do, provided that it is carried out in the period between the beginning of July and the end of September (JEPPSON *et al.* 1958).

Table VII. *Mites and recommended dosages for control*

Mite		Recommended dosages		
		In U. S. units		In metric units
Genus and species	Common name	4 E [a] (pt./100 gal.)	25 WP (lb./100 gal.)	Active ingredient (g./100 l.)
Aceria sheldoni Ewing	citrus bud mite	1/2—3/4	1—1 1/2	30—45
Phyllocoptruta oleivora Ashmed	rust mite.	1/4—1/2	1/2—1	15—30
Aculus pelekassi Keifer	rust mite	1/4—1/2	1/2—1	15—30
Brevipalpus lewisi McGregor	flat mite	1/2	1	30
Panonychus citri McGregor	citrus red mite	1/2	1	30
Eutetranychus banksi McGregor	Texas citrus mite	1/2	1	30
Eotetranychus sexmaculatus Riley	six-spotted mite	1/2	1	30
Eotetranychus yumensis McGregor	Yuma spider mite	1/2—3/4	1—1 1/2	30—45
Tetranychus pacificus McGregor	Pacific spider mite	1/2—3/4	1—1 1/2	30—45
Tetranychus cinnabarinus Boisduval	—	1/2	1	30

[a] 4 E = 45.5 percent of active ingredient.

The product is also effective against *Aceria sheldoni*. The results of trials in which several products were compared always showed that chlorobenzilate was the most efficient product against this mite of the family Eriophyidae (LE PELLEY 1955, JEPPSON *et al.* 1957 a and 1958, SCHWARTZ and RIEKERT 1967).

Brevipalpus lewisi, a mite damaging fruits in California, could be well controlled with chlorobenzilate (dosage 10 pt. of 25 E in 250 gal./acre), the product being far more effective than the organic phosphates tested in the same trials (ELMER and JEPPSON 1957). SCHWARTZ and RIEKERT (1967) see the advantage of chlorobenzilate over organic phosphates in the fact that it controls the mite population in citrus for a longer period of time than do the latter. This is due to the fact that chlorobenzilate is non-toxic to useful insects. Hence, it may be usefully applied in integrated pest control schemes.

3. Field and vegetable crops. — Cotton, beans, eggplants, melons, cucumbers, and tomatoes (Table VIII).

Table VIII. *Mites and recommended dosages for control*

Mite		Recommended dosages		
		In U. S. units		In metric units
Genus und species	Common name	4 E [a] (qt./acre)	25 WP (lb./acre)	Active ingredient (kg./ha.)
Tetranychus urticae Koch	two-spotted spider mite	1/4–1	1–4	0.28–1.1
Tetranychus atlanticus McGregor	strawberry spider mite	1/4–1	1–4	0.28–1.1
Tetranychus pacificus McGregor	Pacific spider mite	1/4–1	1–4	0.28–1.1
Tetranychus schoenei McGregor	Schoene spider mite	1/4–1	1–4	0.28–1.1
Tetranychus cinnabarinus Boisduval	—	1/4–1	1–4	0.28–1.1
Panonychus ulmi Koch	European red mite	1/4–1	1–4	0.28–1.1

[a] 4 E = 45.5 percent of active ingredient.

Cotton is exclusively attacked by tetranychid mites, usually by several species at the same time. Chlorobenzilate is well suited for the control of these mites, even if they have developed resistance to phosphates (LEIGH 1962). Its initial action compared with that of the phosphates is slightly shorter (a 90 percent mite decrease is not obtained before eight days after treatment), but it remains active up to six weeks (MISTRIC 1964, LEIGH *et al.* 1967). The dosage recommended is 0.28 to 1.1 kg. a.i./ha. Also, in this case, thorough wetting of all the plant parts (particularly underleaf) is essential. The treatment is to be carried out at the initiation of the first mite attack (symptoms: reddish spots develop on the leaves); no treatment must be carried out after opening of the bolls.

Beans, eggplants (BASU and PRAMANIK 1968), cucumbers, and melons are attacked by *Tetranychus urticae* and can be successfully treated at 0.125 to 0.5 kg. a.i./ha. or 25 to 50 g. a.i./100 l., respectively. Against the acarinosis of the tomato (agent: *Aculus lycopersici* Massée) a chlorobenzilate treatment at 30 g. a.i./100 l. is enough (BLANCK *et al.* 1956).

4. Tea. — See Table IX for recommendations. Tea is attacked by *Tetranychidae, Eriophyidae,* and *Phytoptipalpidae;* of greatest economic significance, however, is the tea red spider mite. Since 1955 chlorobenzilate has been used in India for the control of mites. Three applications of 25 g. a.i./100 l. resulted in good control of scarlet mites (BAPTIST and RANAWEERA 1955). ANANTHAKRISHNAN (1961/62) confirms the good knock-down and residual action of the product in the control of *Oligonychus coffaeae, Calacarus carinatus,* and *Brevipalpus obovatus.* The treatments must be carried

Table IX. *Mites and recommended dosages for control*

Mite		Recommended dosages		
Genus and species	Common name	50 ES (%)	25 ES (%)	Active ingredient (g./100 l.)
Tetranychidae				
Oligonychus coffeae Nietner (= *Metatetranychus bioculatus* W.-M.)	tea red spider	0.075–0.1	0.15–0.2	37.5–50
Eriophyidae				
Calacarus carinatus Green	ribbed tea mite or purple mite	0.075–0.1	0.15–0.2	37.5–50
Eriophyes theae Watt.	pink tea mite	0.075–0.1	0.15–0.2	37.5–50
Phytoptipalpidae				
Brevipalpus obovatus Donnadieu (= *B. australis,* Tucker)	scarlet mite	0.075–0.1	0.15–0.2	37.5–50
Brevipalpus phoenicis Geijskes	scarlet mite	0.075–0.1	0.15–0.2	37.5–50
Brevipalpus californicus Banks	scarlet mite	0.075–0.1	0.15–0.2	37.5–50

out with the high-volume method. Upper and under surface of the tea leaves must be thoroughly wetted, so that all the mites come into contact with the spray and that no new build-up of the population by remaining mites is possible. According to Ranaweera (1958) 1,680 l. of spray liquid/ha. are necessary for a good success. In the case of heavy infestation the treatment should be repeated after seven days.

Chlorobenzilate has the great advantage that — even immediately after application — it does not impair the flavour or the taste of the tea.

5. Ornamental plants. — Roses, conifers, and shrubs (Table X). Applied at the recommended dosages chlorobenzilate is effective against mites on ornamentals. Roses that are frequently attacked by *Tetranychus urticae* are to be treated with 30 g. a.i./100 l. only, on account of potential damage. According to Wolfenbarger (1964) no damage occurred even after application of 60 g. a.i./100 l. A strain of *T. urticae* that has developed resistance to phosphates and some specific acaricides, among them also chlorobenzilate, could be successfully controlled on greenhouse roses with the combination azobenzene + chlorobenzilate at the rate of 126 g. a.i. + 22.5 g. a.i./100 l. (Jefferson and Morishita 1958). For the complete elimination of the privet mite, a mite on numerous ornamentals, only 20 g. a.i./100 l. were necessary (Morishita 1954).

Table X. *Mites and recommended dosages for control*

Mite		Recommended dosages		
		In U. S. units		In metric units
Genus and species	Common name	4 E [a] (pt./100 gal.)	25 WP (lb./100 gal.)	Active ingredient (g./100 l.)
Tetranychus urticae Koch	two-spotted spider mite	1/2–3/4	1–1 1/2	30–45
Oligonychus ununguis Jac.	spruce spider mite	1/2–3/4	1–1 1/2	30–45
Brevipalpus obovatus Donnadieu (= *B. inornatus* Banks)	privet mite	1/2–3/4	1–1 1/2	30–45

[a] 4 E = 45.5 percent of active ingredient.

c) Control of the acarine disease in bees

Since 1953 chlorobenzilate has been successfully used against the tracheal mite of the bee, *Acarapis woodi* Rennie, on account of its specifically acaricidal action (GUBLER *et al.* 1953). Smouldering Folbex Fumigant Strips (filter paper strips impregnated with 0.5 g. of active ingredient) are put into a sealed bee-hive. The deposit left by the fumes containing chlorobenzilate in the hive, on the bees, and in their tracheae acts as a contact acaricide. In contradistinction to the traditional gases which are used for the control of the acarine disease and only possess immediate but no residual action, chlorobenzilate remains effective over a longer period of time (SCHNEIDER 1959).

Folbex deposits remain active during 14 days in the hairs of bees and during four weeks in their tracheae. A single Folbex treatment protects young bees from a reinfestation for at least eight days. For a complete recovery of a bee colony from acarine disease, eight treatments at weekly intervals are necessary. It is known from practice that a Folbex application consisting of eight successive treatments did not impair the flavour and the taste of honey (DREHER *et al.* 1959).

d) Phytotoxicity

Chlorobenzilate is well tolerated by *pome fruits.* Damage on apples of the varieties Delicious, Jonathan, McIntosh, and pears of the variety Comic are known from the U.S.A. only where relatively high dosages (30 to 45 g. a.i./100 l., treatment repeated after seven to 10 days) are recommended in fruit growing. Various *stone fruit varieties* (mainly peaches and plums), however, are less tolerant to chlorobenzilate applications (two treatments at 25 g. a.i./100 l. at an interval of three to four weeks). On the leaves of most peach trees there were symptoms of phytotoxicity, *viz.,* small-to-large red spots which sometimes extended over more than 30 percent of the leaf

surface, according to the variety. Leaf drop may occur on highly susceptible varieties. Plums and prunes, too, are more or less susceptible according to the variety. Three out of ten varieties tested, namely Bleu de Belgique, Reine Claude d'Oullins, and Fellenberger Zwetschge, reacted with leaf injury. After two treatments at 25 and 30 g. a.i./100 l. (WP and ES formulation) only two out of six cherry varieties, i. e., Bigarreau Moreau and Bigarreau Jaboulay, showed slight leaf yellowing, which disappeared after 45 days, though. According to further investigations peaches, apricots, and numerous cherry varieties tolerated 45 g. a.i./100 l. of a 25 percent WP formulation without injury (WOLFE 1955).

Chlorobenzilate is usually well tolerated by *grape vines*. In Argentina, however, cases of injury on the varieties Tokayer, Merlot, Verdot, and Barbera de Asti occurred after preblossom sprays.

Ornamentals are generally not susceptible to chlorobenzilate. For treatments of roses no more than 30 g. a.i./100 l. may be used. Out of 13 foliage trees tested only *Acer saccharum* and *Alnus glutinosa* were badly injured after a three percent chlorobenzilate spray. No or only slight damage occurred on *Quercus borealis, Manolia glauca, Viburnum lantana, Malus sp., Syringa sp., Pinus strobus, Cercis canadensis,* even at a 30-fold overdosage like this (CLOWER and MATHYSSE 1954). Neither the aspect of the leaves nor the flavour of the *tea* are impaired by chlorobenzilate treatments (BAPTIST and RANAWEERA 1955).

Also *cotton* (ATTIAH 1958) and *citrus* are non-susceptible to chlorobenzilate.

VI. Trademarks and formulations of chlorobenzilate

These are shown in Table XI.

Table XI. *Trademarks and formulations of chlorobenzilate*

Commercial designation	Technical chlorobenzilate (%)	Formulation
AKAR 338, KOPMITE	25	wettable powder
AKAR 20	20	emulsifiable solution
AKAR 338, GESASPIND, GEIGY 338	25	emulsifiable solution
ACARABEN 4 E	45.5	emulsifiable solution
AKAR 50	50	emulsifiable solution
FOLBEX	40	fumigant strip

VII. Residues of chlorobenzilate

a) Residues from supervised trials

Pome fruits, shell fruits, citrus, melons, vinegrapes, hops, tea, and cottonseed were analyzed for residues of chlorobenzilate after treatments at vary-

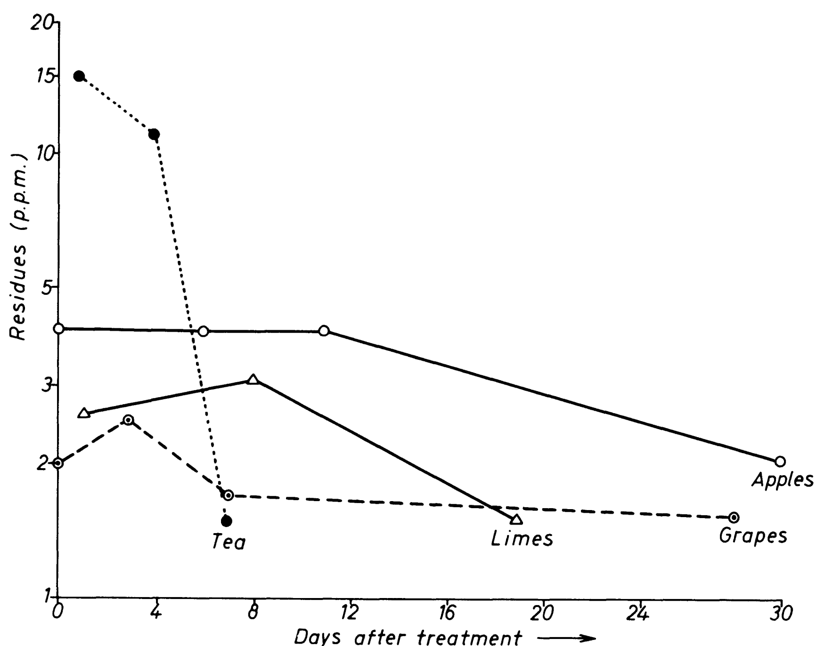


Fig. 1. Dissipation curves of chlorobenzilate: apples ○——○, four sprays with 60 g. of a. i./100 l.; limes △——△, one spray with 60 g. of a. i./100 l.; grapes ⊙——⊙, two sprays with 60 g. of a. i./100 l.; and tea ●——●, one spray with 12.5 g of a. i./100 l.

ing doses. Table XII presents a number of analysis results obtained by ultraviolet, colorimetric, or gas chromatographic methods in the U.S.A. and in Switzerland. In Figure 1 some results display the dissipation of chlorobenzilate in a graph.

With the exception of some residue data from the year 1952 on *apples*, already the initial residues (active ingredient deposits immediately after last treatment) are below the level of five p.p.m. which has been established as a tolerance for pome fruit in some countries, and as a temporary tolerance by FAO/WHO. On *pears*, only slight active ingredient deposits are observed; eight days following the third treatment only 0.46 p.p.m. of chlorobenzilate is present.

Hardly any residues (measured maximum value <0.25 p.p.m. immediately after treatment) are found in the utilisable parts of *shell fruit* (walnuts and almonds). The greater part of the active ingredient remains on the hulls, the outer covering of the fruit, whereas only 15 percent of the total chlorobenzilate residues is found on the shells.

With *citrus* (lemons, grapefruits, tangerines, oranges, and limes), chlorobenzilate has only been found in the peel where it is very slowly degraded. On the strength of analysis by ultraviolet and colorimetric methods,

GUNTHER *et al.* (1955) give a half-life of 60 to 80 days for chlorobenzilate degradation in lemon peel under field conditions. Gas-chromatographic analysis provide a half-life for chlorobenzilate degradation in the peel of limes of about 20 days (compare Table XI).

On *vine grapes*, the initial residues are between two and three p.p.m. where they are also very slowly degraded. Irrelevant to the treatment dosage they are 1.25 p.p.m. after four weeks. On *melons* and *hops* only very small amounts of active ingredient are found.

Dried *tea* leaves have high residue levels one to three days after treatment. Yet, chlorobenzilate disappears quickly from tea leaves; already one week after treatment only 10 to 50 percent of the initial residues are present. The process of drying seems to have an influence on the residue level. Hence, about 80 percent less chlorobenzilate is found on the sun-dried leaves than on the manufactured dried leaves. Also the further manufacturing process has a great influence on the final chlorobenzilate residue level. Made tea has only about 10 to 30 percent of the residue found on dried tea leaves. When tea was being brewed, chlorobenzilate was mainly found in the wet leaves, whereas merely 0.06 p.p.m. was found in the liquid (see Table XI).

No chlorobenzilate can be found in *cottonseed* 93 and 70 days, respectively, after last treatment.

b) Behaviour and fate of the residues

On apples and citrus fruits chlorobenzilate is found exclusively in the peel where it disappears only slowly, as Figure 1 shows. According to studies by MURPHY *et al.* (1966), the chlorobenzilate applied on apples remains unchanged in the apple peel and does not penetrate into the meat. None of the treated apples contained detectable amounts of 4,4′-dichlorobenzilic acid [1]. Also, on citrus fruits chlorobenzilate is only found in the peel (compare Table XII and GUNTHER *et al.* 1955). No special studies on a chemical change of the active ingredient in citrus peels are available. But on the strength of studies on the behaviour of chlorobenzilate on soybean leaves and tea, it can be assumed that chlorobenzilate is not metabolized in citrus peels either.

HASSAN and KNOWLES (1969) have studied the behaviour and the fate of C^{14}-labelled chlorobenzilate on soybean leaves. They found that after the application chlorobenzilate rapidly penetrated into the leaf tissues and was translocated from there unchanged into the petioles after about 12 days.

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[1] Apple trees were sprayed with two lbs. of chlorobenzilate a.i./100 gal.; samples were taken three, 14, and 21 days after application. The sensitivity of the analytical method was 0.1 p.p.m. The recovery values indicate the ability to detect the 4,4′-dichlorobenzilic acid with no interference from chlorobenzilate. Extraction studies on field-weathered 14-day samples showed that unchanged chlorobenzilate was present at 90 to 94 percent on the surface of the apple, and not in the meat. Chlorobenzilate injected into the apple meat (two mg. a.i., dissolved in hexane) was recoverable at 86 percent and also unchanged after 16 days.

In culture media, chlorobenzilate is metabolized by microorganisms. Yeast (*Rhodotorula gracilis* Rennerfelt) particularly is able to degrade chlorobenzilate in two main and some intermediate metabolites in a mineral medium supplemented by sucrose. The conversion is presumably due to hydrolysis to 4,4′-dichlorobenzilic acid and then to decarboxylation, as well as dehydrogenation to 4,4′-dichlorobenzophenone. Not even after an eight-week incubation could chlorobenzilate be completely metabolized. This incomplete degradation would appear to be due to storage of the apolar compound in fat of the yeast cells, where litte enzymatic degradation would occur (MIYAZAKI *et al.* 1970).

c) Flavour data

Numerous flavour tests have been conducted on crops treated with chlorobenzilate. In fresh apples and applesauce, there was only one sample reported different from the check, and this one was acceptable, but different due to difference in maturity. Tests made on fresh and canned peaches, canned pears, cantaloupes, and orange juice indicated that all samples were not different from the check, except that juice from treated Valencia oranges was more acid than that from the checks. There was no general objection to the acid flavour. The present residue data would hardly account for off-flavour of fruit meat and fruit juice since chlorobenzilate could in no case be found in the meat of apples, citrus, and melons. Equally unimpaired are flavour and taste of brewed tea in which chlorobenzilate — even in traces — is still detectable.

d) Residues in food at the time of consumption

In "market basket" or "total diet" studies carried out by the *U. S. Food and Drug Administration*, multidetection methods for residue analysis were used. The analytical procedure used for all samples in this study enable the detection of about 54 pesticide chemicals, including chlorobenzilate. So far, no residues of chlorobenzilate have been found (CUMMINGS 1966).

e) Tolerances and waiting periods

Tolerances for chlorobenzilate have been established in the countries shown in Table XIII.

Temporary tolerances (to be in effect until 1972) were proposed by FAO/WHO in 1968 for the raw agricultural products moving in commerce shown in Table XIV.

Waiting periods (days between last application and harvest) for chlorobenzilate applications have been recommended in the U.S.A. and in France. In the U.S.A. chlorobenzilate should not be applied to apples within 14 days, or to pears within seven days of harvest. In France chlorobenzilate should not be applied within seven days of harvest.

Table XII. *Chlorobenzilate residues in various crops*

Crop and variety	Country and year	Ref.[a]	Dosage and formulation[b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method[c]	Residues (p. p. m.) and plant parts analyzed
								fruits
Apples	U.S.A. N. Jersey (1952)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	12. VI.	1	0	UV	9.73
			1 lb. of 25 EC 30 g. a.i./100 l.	12. VI.	1	0	UV	16.99
			1 lb. of 25 WP 30 g. a.i./100 l.	18. VII./25. VII.	2	7	UV	4.81
			2 lb. of 25 WP 60 g. a.i./100 l.	18. VII./25. VII.	2	7	UV	7.92
			1 lb. of 25 EC 30 g. a.i./100 l.	18. VII./25. VII.	2	7	UV	3.40
Apples	U.S.A. N. York (1953)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	14. VIII.	1	33	UV	3.84
				14. VIII.	1	33	UV	0.00
			1/2 lb. of 25 WP 15 g. a.i./100 l.	15. VI./30. VI./15. VII./ 30. VII./12. VIII.	5	56	UV	2.62
Apples Red Delicious	U.S.A. Oregon (1967)	G.A.	2 lb. of 25 WP 60 g. a.i./100 l.	29. VI./13. VII./1. VIII./ 16. VIII.	4	0	GC	4.0
						0		4.0
						6		5.3
						6		2.8
						13		4.8
						13		3.2
						30		1.9
						30		2.1
						42		1.8
						42		1.9

Pears	U.S.A. Calif. (1953)	G.A.	2 lb. of 25 WP 60 g. a.i./100 l.	21. VI./18. VII./19. VIII.	3	8	UV		0.53	
						8			0.36	
						8			0.51	
		G.A.	2 lb. of 25 WP 60 g. a.i./100 l.	9. VI./4. VII.	2	60	UV		0.14	
						60			0.08	
						60			0.11	
						60			0.02	
Pears Bartlett	U.S.A. Calif. (1953)	G.A.	1½ lb. of 25 WP 45 g. a.i./100 l.	26. V./19. VI.	2	31	UV		0.37	
						31			0.38	
						31			0.42	
									shell	*meat*
Walnuts Hartley	U.S.A. Calif. (1966)	G.A.	1) 0.76 lb. a.i. of 4 E 0.85 kg. a.i./ha.	1) 19. VII.	2	28	GC		< 0.1	< 0.1
			2) 2.25 lb. a.i. of 4 E 2.5 kg. a.i./ha. +surfactant X-77 at 4 oz./100 gal.	2) 30. VIII. before husk split spray mixture: 200 gal./acre		28			< 0.1	< 0.1
		G.A.	1) 1.5 lb. a.i. of 4 E 1.68 kg. a.i./ha.	1) 19. VII.	2	28	GC		< 0.1	< 0.1
			2) 4.5 lb. a.i. of 4 E 5.0 kg. a.i./ha. +surfactant X-77 at 4 oz./100 gal.	2) 30. VII. before husk split spray mixture: 200 gal./acre		28			< 0.1	< 0.1

[a] References: G.A. = *Geigy Chemical Corp.*, Ardsley (N. Y.): Residue reports, unpublished.
G. = GUNTHER, F. A., L. R. JEPPSON, and G. B. WACKER: Persistence of chlorobenzilate residues in mature lemon fruits. J. Econ. Entomol. **48**, 372 (1955).
G.B. = *J. R. Geigy AG.*, Basel: Residue reports, unpublished.

[b] Formulation: WP = wettable powder, EC = emulsifiable concentrate, and E = emulsifiable concentrate.

[c] Analytical method: UV = ultraviolet, GC = gas chromatographic, and Col. = colorimetric.

[d] Treated leaves (389 kg.) were mixed with untreated leaves (2,106 kg) to afford a 1 : 5.4 mixture.

Table XII (continued)

Crop and variety	Country and year	Ref.[a]	Dosage and formulation[b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method[c]	Residues (p. p. m.) and plant parts analyzed		
								hulls	*shells*	*nuts*
Almonds Nonpareil	U.S.A. Calif. (1966)	G.A.	0.75 lb. of 4 E 45 g. a.i./100 l.+oil	3. VII. 27. VII. before hull split spray mixture: 500 gal./acre	2	0	GC	15.0	2.2	0.24
						5		14.0	1.8	0.14
						8		20.0	2.1	0.20
						13		6.0	0.94	0.11
						29		14.0	1.5	0.12
						43 (harvest)		12.0	0.57	< 0.1
Almonds Mission	U.S.A. Calif. (1966)	G.A.	0.75 lb. of 4 E 45 g. a.i./100 l.+oil	3. VII. 27. VII. before hull split spray mixture: 500 gal./acre	2	0	GC	23.0	3.1	0.12
						5		27.0	3.3	0.11
						8		19.0	2.8	0.10
						13		16.0	1.7	< 0.10
						29		9.8	0.81	< 0.10
						64 (harvest)		1.4	< 0.10	< 0.10
Almonds Nonpareil, Neplus, Mission } mixed	U.S.A. Calif. (1966)	G.A.	0.75 lb. of 4 E 45 g. a.i./100 l.+oil	20. VII. fruit fully developed hulls not split spray mixture: 400—600 gal./acre	1	0	GC	0.74	0.45	< 0.10
						0		0.72	0.50	< 0.10
						2		0.44	0.26	< 0.10
						2		0.31	0.25	< 0.10
						5		1.1	0.20	< 0.10
						5		0.75	0.24	< 0.10
						19		0.66	0.43	< 0.10
						19		0.90	0.34	< 0.10
						33		1.2	0.29	< 0.10
						33		2.0	0.36	< 0.10
						63		3.0	0.53	< 0.10
						63		1.5	0.62	< 0.10

									peel
Lemons	U.S.A. Calif. (1954)	G.	1 lb. of 25 WP 30 g. a.i./100 l.	31. III. spray mixture: 1,000 gal./acre	1	1	UV		10.7
						2			8.5
						14			8.6
						28			8.3
						60			5.7
						85			4.8
						113			3.5
						140			2.6
		G.	1 lb. of 25 WP 30 g. a.i./100 l.	31. III. spray mixture: 1,000 gal./acre	1	1	Col.		9.5
						2			9.5
						14			9.8
						28			8.9
						60			6.2
						85			4.8
		G.	3 lb. of 25 WP 90 g. a.i./100 l.	31. III. spray mixture: 1,000 gal./acre	1	1	UV		16.9
						2			19.0
						14			17.9
						28			19.6
						60			13.9
						85			12.8
						113			8.5
						140			7.0
		G.	3 lb. of 25 WP 90 g. a.i./100 l.	31. III. spray mixture: 100 gal./acre	1	1	Col.		14.9
						2			16.7
						14			19.0
						28			19.1
						60			13.9
						85			11.7
								peel	*pulp*
Grapefruits	U.S.A. Florida (1957)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	—	1	6	UV	1.10	—
						11		2.70	0.00

Table XII (continued)

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p. p. m.) and plant parts analyzed	
								peel	*pulp*
Grapefruits	U.S.A. Arizona (1957)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	—	1	0	UV	1.12	
						0		1.08	
						3		1.89	
						7		1.84	
						14		1.38	0.00
						21		2.14	
Tangarines	U.S.A. Florida (1957)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	—	1	6	UV	3.77	0.00
						11		3.47	
	U.S.A. Arizona (1957)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	—	1	0	UV	1.68	
						0		1.12	
						3		1.99	
						7		1.99	0.00
						14		1.88	0.00
						21		2.39	
						34		3.06	
Oranges Sweet	U.S.A. Arizona (1957)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	—	1	0	UV	1.22	0.00
						3		1.17	
						7		1.63	0.00
						14		2.04	
						21		1.32	
						34		0.97	0.00
Oranges Valencia	U.S.A. Arizona (1957)	G.A.	1 lb. of 25 WP 30 g. a.i./100 l.	—	1	0	UV	1.38	
						3		1.78	
						7		2.80	0.00
						14		1.43	
						21		1.99	

								peel	pulp
Limes	U.S.A. Florida (1962)	G.A.	1 lb. of 25 WP 30 g. a.i./100 gal.	7. VI. spray mixture: 400 gal./acre	1	1	GC	2.6	0.1
						1		2.5	—
						8		1.2	< 0.1
						19		1.2	< 0.1
						19		1.5	< 0.1
		G.A.	2 lb. of 25 WP 60 g. a.i./100 gal.	7. VI. spray mixture: 400 gal./acre	1	1	GC	2.6	< 0.1
						1		2.5	—
						8		3.0	< 0.1
						8		3.1	—
						19		1.5	< 0.1
						19		1.4	—
									fruit
Grapes Thompson seedless	U.S.A. Calif. (1966)	G.A.	1 lb. of 4 E 60 g. a.i./100 gal.	5. VIII./2. IX. spray mixture: 200 gal./acre growth stage of the fruit: mature	2	0	GC		2.4
						0			1.7
						3			2.1
						3			3.4
						7			1.9
						7			1.5
						17			0.88
						17			0.37
						28			0.81
						28			1.3
		G.A.	2 lb. of 4 E 120 g. a.i./100 gal.	5. VIII./2. IX. spray mixture: 200 gal./acre growth stage of the fruit: mature	2	0	GC		3.1
						0			6.3
						3			1.5
						3			1.1
						7			3.3
						7			0.44
						17			1.4
						17			1.0
						28			1.1
						28			1.2

Table XII (continued)

Crop and variety	Country and year	Ref.[a]	Dosage and formulation[b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method[c]	Residues (p. p. m.) and plant parts analyzed		
									fruits	
Melons Crenshaw	U.S.A. Calif. (1964)	G.A.	4 lb. of 25 ES 1.12 kg. a.i./ha.	foliage spraying 12. X. spray mixture: 100 gal./acre	1	1	GC		< 0.04	
						7			0.16	
						14			< 0.04	
						22			< 0.04	
Melons Casaba	U.S.A. Calif. (1964)	G.A.	4 lb. of 25 ES 1.12 kg. a.i./ha.	foliage spraying 12. X. spray mixture: 100 gal./acre	1	1	GC		0.25	
						7			0.06	
						14			0.18	
						22			0.17	
								whole fruit	*rind*	*meat*
Muskmelons	U.S.A. Florida (1965)	G.A.	2 lb. of 25 WP 0.56 kg. a.i./ha.	broadcast over the top 9. VIII. spray mixture: 50 gal./acre	1	0	GC	0.31		
						0		0.13		
						7		< 0.10		
						7		< 0.10		
						14		< 0.10	< 0.1	< 0.1
						14		< 0.10		
		G.A.	8 lb. of 25 WP 2.24 kg. a.i./ha.	broadcast over the top 9. VIII. spray mixture: 50 gal./acre	1	0	GC	1.8		
						0		0.9		
						7		0.66		
						7		1.20		
						14		< 0.10	0.14	< 0.1
						14		< 0.10		

Hops Yakima cluster	U.S.A. Wash. (1966)	G.A.	3.6+1.8 lbs. of 4 E 2+1 kg. a.i./ha.	21. VII./11. VIII. spray mixture:	2		GC	*green*	*dry*	
				1) 150 gal./acre		29		< 0.10	< 0.10	
						29		0.11	< 0.10	
				2) 150 gal./acre		28		0.13	1.5	
						28		0.09	1.3	
			3.6+1.8+1.8 lbs. of 4 E 2+1+1 kg. a.i./ha.	21. VII./11. VIII./24. VIII. spray mixture:	3	16	GC	1.1	0.12	
				1) 150 gal./acre		16		0.94	< 0.10	
				2) 150 gal./acre		16		0.62	1.2	
				3) 225 gal./acre		16		0.74	1.3	
									dried leaves	*ferment-ed leaves*
Tea	Indonesia Java (1968)	G.B.	0.025% of 50 ES 12.5 g. a.i./100 l.	spray mixture: 400 l./ha. corresp. to 50 g. of a.i./ha.	1	1	GC		17.0	1.4
						1			13.0	
						4			12.0	1.7
						4			10.0	
						7			1.5	< 0.20
						7			1.4	
						9			—	
						9			—	0.63
		G.B.	0.05% of 50 ES 25 g. a.i./100 l.	100 g. of a.i./ha.	1	1	GC		9.1	5.60
						1			18.0	
						4			21.0	5.50
						4			16.0	
						7			7.6	4.10
						7			6.1	
						9			—	5.30
						9			—	
Tea Albizzia odoratissima	India Assam (1969)	G.A.	1.25 l. of 25 ES/ha. 312 g. of a.i./ha.	spray mixture: 100 l./ha.	1	7	GC		*manufactured tried leaves*	
									unmixed	*mixed* [d]
									45.6	< 0.1

Table XII (continued)

Crop and variety	Country and year	Ref.[a]	Dosage and formulation[b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method[c]	Residues (p. p. m.) and plant parts analyzed		
									brewed tea *wet leaves*	*brew*
									37.4	0.06
Cottonseed Undelinted	U.S.A. Calif. (1962)	G.A.	4 lb. of 25 ES 1.12 kg. a.i./ha.	20. VI./11. VII. ground spray	2	93	GC		< 0.10	
		G.A.	4 lb. of 25 ES 1.12 kg. a.i./ha.	13. VII./1. VIII./16. VIII. aerial application	3	70	GC		< 0.10	

Table XIII. *Tolerances for chlorobenzilate*

Country	Raw agricultural product	Tolerances (p.p.m.)
Canada	apples, pears, citrus fruits, cantaloupes	8
The Netherlands	fruits, vegetables	1.5
New Zealand	fruits, vegetables	5
South Africa	fruits	5
United States	apples, pears, citrus fruits, melons	5
	almonds, walnuts	0.2
	almond hulls	15
	cottonseed	0.5
	meat, fat and meat, by-products of cattle and sheep	0.5

Table XIV. *FAO/WHO temporary tolerances for chlorobenzilate*

Raw agricultural product	Temporary tolerances (p.p.m.)
Apples, pears (whole-fruit basis)	5
Citrus (whole-fruit basis)	1
Melons, cantaloupes	1
Almonds, walnuts	0.2

VIII. Analysis of chlorobenzilate

a) Analysis of active ingredient and formulations

1. Review of methods. — Technical chlorobenzilate may be analyzed by determination of total chlorine, as described by MEYER and SUTER (1953) and LARSEN *et al.* (1970). The same authors used the saponification of the ester group and, especially for composition analysis, the acetylation of the hydroxy group. HENDRIX (1969) describes an infrared method for the determination of chlorobenzilate.

The purity of technical chlorobenzilate may also be determined by gas chromatography, both by total area and internal standard methods (BAILY and CURRY, 1965, LARSEN *et al.* 1970). The latter procedure is highly recommended for the analysis of the active ingredient and formulations.

2. Analysis of technical chlorobenzilate. —

α) *Total chlorine content.* —

APPARATUS:

200-ml. ground joint Erlenmeyer flask
Reflux condenser, with ground glass joint

REAGENTS:

Metallic sodium
Isopropyl alcohol, redistilled, free of organic chlorine
Isopropyl alcohol, 70 percent in water
Sulfuric acid, pure, 50 percent
Silver nitrate solution, 0.1 *N*

PROCEDURE:

Weigh 0.28 to 0.30 g. of chlorobenzilate into a 200-ml. ground joint Erlenmeyer flask, add 50 ml. of isopropanol and about 5 g. of sodium metal, cut into small cubes. Connect with dry reflux condenser and keep slightly boiling for at least one hour. Destroy the excess sodium metal by introducing, drop by drop, a few ml. of the 70 percent isopropanol through the condenser. Boil again for a few minutes and add 50 ml. of distilled water. After cooling, transfer the solution into a 250-ml. beaker and rinse the Erlenmeyer flask with a few ml. of water. Acidify slightly with 50 percent sulfuric acid and titrate potentiometrically with 0.1*N* silver nitrate solution, using electrodes Ag and Hg/Hg_2SO_4 with a K_2SO_4 bridge.

ml. 0.1 *N* silver nitrate solution consumed = a
weight of sample in g. = w

Calculation: $$\frac{a \times 325.19 \times 100}{w \times 20{,}000} = \text{percent chlorobenzilate}$$

Ionic chlorine has to be determined separately as follows and subtracted in the calculation:

Dissolve a three to six g. sample of chlorobenzilate in 50 ml. of isopropanol, dilute with 50 ml. of water, acidify with 50 percent sulfuric acid, and titrate as above.

β) Saponification. —

APPARATUS:

200-ml. ground joint Erlenmeyer flask
Reflux condenser with ground glass joint

REAGENTS:

Neutral ethanol
Potassium hydroxide, 0.5 *N* solution in ethanol
Hydrochloric acid 0.5 *N* solution
Phenolphthalein 0.1 percent solution in 60 percent ethanol

PROCEDURE:

Weigh four to five g. of chlorobenzilate into a 200-ml. ground joint Erlenmeyer flask, and add 50 ml. of neutral ethanol. Neutralise, after addition of some drops of phenolphthalein solution, with 0.5*N* ethanolic potas-

sium hydroxide solution to red. Add 50.0 ml. of 0.5*N* ethanolic potassium hydroxide solution and reflux for one hour. Rinse the condenser with a few ml. of neutral ethanol. Without cooling, back-titrate the excess potassium hydroxide solution with 0.5*N* hydrochloric acid, using phenolphthalein as indicator.

ml. 0.5 *N* hydrochloric acid consumed = a
weight of sample in g. = w

In the same way run a blank test with 50.0 ml. of 0.5*N* ethanolic potassium hydroxide solution and titrate with 0.5*N* hydrochloric acid as before:

ml. 0.5 *N* hydrochloric acid consumed = b

Calculation: $\frac{(b-a)\times 325.19\times 100}{w\times 2{,}000}$ = percent chlorobenzilate

γ) Gas chromatography (internal standard method). —

RECOMMENDED INSTRUMENT PARAMETERS:

Instrument: Aerograph, model 1200-1
Integrator: Infotronics, model CRS-104
Recorder: Sargent, 2.5 mV, chart speed 1.2 cm./minute
Column: glass, 1 m.×3.5 mm. with 5 percent OV 25 silicone on Chromosorb W AW DMCS HP 80/100 mesh
Detector: flame ionization detector
Temperatures: *column oven* — 210 °C. isothermal
detector — 210 °C.
injector — 240 °C.
Carrier gas: helium — 60 ml./minute
hydrogen — 60 ml./minute
air — 600 ml./minute
Sample solution: 1 g. of chlorobenzilate and 1 g. of internal standard (succinic acid dibenzylester) both analytically weighed are dissolved in 10 ml. of methylene chloride
Volume injected: 0.5 µl.
Relative retention times: chlorobenzilate — 1.00
standard — 1.62
Total time for chromatogram: 20 minutes
Recorder set: sensitivity adjusted to give 60 to 80 percent response

CALCULATIONS:

$$\%\ \text{chlorobenzilate} = \frac{G_{St}\cdot F_A\cdot f\cdot 100}{G_A\cdot F_{St}}$$

G_{St} = weight of standard in g.
G_A = weight of chlorobenzilate in g.
F_{St} = peak area of standard in counts
F_A = peak area of chlorobenzilate
f = specific factor, numerical value about 1.2; has to be determined at each analysis with a reference sample

Precison: ± 1 percent at 95 percent confidence limits for duplicates.

3. Analysis of formulations. —

α) Principle. — Chlorobenzilate in emulsifiable solutions and wettable powders is analyzed by the determination of the total chlorine content. In the case of wettable powders the active ingredient is extracted by ethyl ether, whereas emulsions can be directly analyzed. The emulsifiable solution or the ether extract of a wettable powder formulation is dehalogenated with metallic sodium (STEPANOW 1906, UMHOEFER 1943, CARTER and HUBANKS 1946, CARTER 1947, CARTER *et al.* 1950).

β) Procedure. — Weigh accurately an amount of product containing between 0.28 and 0.30 g. of chlorobenzilate and extract in a Soxhlet apparatus with 130 ml. of ethyl ether during eight hours. Evaporate to dryness and dissolve the residue in 50 ml. of isopropanol. Continue as described under "Analysis of technical chlorobenzilate — Total chlorine content — Procedure".

γ) Discussion of method. — The method described is suitable for all formulations. For emulsifiable solutions extraction is not necessary. For wettable powders with a relatively high content in active ingredient combustion with sodium peroxide in the Parr or Wurzschmitt bomb is somewhat more rapid (WURZSCHMITT 1950).

b) Residue analysis

1. Extraction. — Several procedures have been tested and are recommended for extracting chlorobenzilate from various crops. BLINN *et al.* (1964) used petroleum ether (boiling point 60° to 80° C.) for the extraction of chlorobenzilate from citrus fruits. The same solvent (boiling point 30° to 60° C.) was successfully applied by MATTSON and KAHRS (1962) and in the WHO- and FAO-method (1969) for extracting chlorobenzilate from apples, pears, stone fruits, citrus fruits, berries, nuts, vegetables, and cotton. Benzene was recommended for the extraction of chlorobenzilate from apples, pears, peaches, cantaloupes, and strawberries by HARRIS (1955). Stearic acid was added to strip solution aliquots as a means of precaution to minimize any possible losses of chlorobenzilate due to volatilization during the evaporation of solvents. BECKMANN and BEVENUE (1964) improved the extraction method developed by HARRIS for grapes and cottonseed. DELLEY (1957) used diethyl ether as extraction solvent for tea leaves. BAKER and SKERRET (1958) studied the extraction of DDT and chlorobenzilate with carbon tetrachloride in a Soxhlet extractor from plant materials.

2. Cleanup. — Depending on the ultimate determination method chosen, there are several alternatives.

Chlorobenzilate is saponified with ethanolic potassium hydroxide in the extractive mixture to 4,4'-dichlorobenzilic acid. BLINN *et al.* (1954) recommended a selective oxidation of 4,4'-dichlorobenzilic acid to 4,4'-dichlorobenzophenone with alkaline permanganate or chromic anhydride in glacial acetic acid. The interfering materials will also be oxidized into easily removable products.

The 4,4′-dichlorobenzilic acid was separated from any dehydrochlorinated DDT present after hydrolysis by extraction in the cleanup procedure by HARRIS (1955), following nitration. The method developed by DELLEY (1957) was based on the same principle, but DELLEY adapted the cleanup to his special problems.

Chromatographic cleanup methods were investigated to separate chlorobenzilate from DDT by BAKER and SKERRETT (1958). They found it desirable to add a little oxalic acid to prevent losses of insecticide. The stearic acid used by HARRIS (1955) for similar purposes in the determination of chlorobenzilate was found to cause interference in the final colour development.

Column chromatography has been used extensively as cleanup technique. Florisil is applied for cleanup in residue work for chlorobenzilate by BECKMANN and BEVENUE (1964), and MATTSON and KAHRS (1962). The column was washed with benzene followed by 20 percent diethyl ether in benzene. Alumina columns (basic, activity grade V) were used to remove interfering materials in the WHO and FAO procedure (1969). Chlorobenzilate was eluted with hexane/benzene (1:1). Interference from DDT and DDD is eliminated by this cleanup procedure.

3. Methods of determination. —

α) Spectrometric methods. — Two alternative specific methods have been developed by BLINN *et al.* (1954) which are based on the hydrolysis of chlorobenzilate to 4,4′-dichlorobenzilic acid and selective oxidation to 4,4′-dichlorobenzophenone. The latter is determined either by its absorption at 264 nm or by the absorption of its 2,4-dinitrophenylhydrazone derivative at 510 nm. Both methods are reproducibly sensitive to about 15 µg. of chlorobenzilate in admixture with three g. of citrus extractives. The structural similarities of 4,4′-dichlorobenzilic acid and DDT suggested the use of the method of SCHECHTER *et al.* (1945). When the product is nitrated, tetranitrodichlorobenzophenone is produced, which reacts with sodium methylate to give a red complex with a maximum absorption at 418 nm and 538 nm. HARRIS (1955) tested the method and modified it for different types of residues and crops. Because of the high purity of organic solvents (without ketone and aldehyde) needed for the BLINN *et al.* (1954) method, the method of HARRIS (1955) is preferred.

In the procedure suggested by ROTH (1958), the stripping solution was directly nitrated and the mixture of DDT and chlorobenzilate is reacted with sodium methylate. The absorption of the coloured solution is measured at two wavelengths. The method has been tried on pears treated in the field. The HARRIS (1955) method was tested and modified by BAKER and SKERRETT (1958) and MARGOT and STAMMBACH (1964).

β) Gas chromatographic methods. — The introduction of several detectors specifically designed for measuring an organohalogen compound that emerges from the gas chromatograph by COULSON *et al.* (1960) simplified the analysis of chlorobenzilate. The application of a gas chromatographic

procedure using a microcoulometric detector for the analysis of residues of chlorobenzilate is reported by BECKMANN *et al.* (1964). The chromatographic column consists of six-foot stainless steel tubing containing 20 percent DOW 11 silicone grease on acid washed Chromosorb P. Replacing the stainless steel tubing with quartz tubing resulted in an improvement in column efficiency. The authenticity of the peak area was verified by infrared spectra of collected fractions compared to the spectrum of reference chlorobenzilate.

A short description of the gas chromatographic determination of chlorobenzilate in fruits, vegetables, and nuts is given by the WHO and FAO method (1969) using a microcoulometric detection system. The glass column was packed with five percent silicone gum GE XE-60 (nitrile) supported on Anakrom (ABS acid and base washed, 50/60 mesh).

The COULSON (1965 and 1966) electrolytic conductivity detector system, normally used for nitrogen detection, was also used successfully to determine halogenated compounds such as chlorobenzilate. The quartz reduction tube was packed with a platinium gauze rolled lengthwise. No absorber was placed in the exit end of the reduction tube. The column was glass, filled with five percent silicone rubber SE 30 on Chromosorb WS.

4. Recommended method. —

α) Principle. — The acaricide is extracted from the crop material by blending with acetone-hexane mixed solvent. The extract is chromatographed to remove the interfering materials. Quantitative measurements are made using a gas chromatograph with a microcoulometric titration cell sensitive to chloride.

REAGENTS:

Acetone: Reagent grade
Benzene: Reagent grade
n-Hexane: BR 65° to 69 °C.
Aluminum oxide: Woelm, basic, activity grade I (Bodman Chemicals, Inc., 106 North Essex Ave., Narberth, Penn. 19072)

Aluminum oxide: Activity grade V. Prepared by mixing 85 g. of Woelm basic alumina, activity grade I with 15 g. of water. The water and alumina are mixed thoroughly and allowed to stand overnight in a tightly closed bottle before use.

Acetonitrile: BR 80.5° to 82.5 °.

APPARATUS:

Chromatographic column for sample cleanup — The column is 18 mm. ID × 200 mm. long and is equipped with a perforated support plate and a 100-ml. reservoir by joining a 100-ml. round-bottom to the top.

Hobart Food Cutter — Catalog no. 84141 (The Hobart Manufacturing Co., Troy, Ohio)

Blender — Osterizer or equivalent

Flash evaporator — Büchi or equivalent

Vials — 2-dram capacity

Microcoulometric gas chromatograph — Model MT 220 (Micro Tek Instruments Corp., Austin, Texas). This instrument is equipped with a Dohrmann (San Carlos, Calif.) T-300-s microcoulometric titration cell sensitive to chloride, a C-200a microcoulometer and an S-200 combustion furnace.

Glass injection port — Micro Tek Instruments Corp. (Austin, Texas)
Syringes — 0.010-ml. sample injection syringe

Gas chromatography column — Glass column (2 feet$\times$1/4 inch O.D.) packed with three percent by weight of Carbowax 20 M on 60/80 mesh Gas Chrom Q.

β) Extraction procedure. — A representative sample of 300 to 400 g. of the crop is chopped in a Hobart Food Cutter. A 100-g. sample is then transferred to a blender jar equipped with a plastic cap. Several untreated check (control) samples are fortified by adding known amounts of the acaricide sought to the samples at this point. The amount added should be near the expected values for the treated samples. A polyethylene liner is placed under the cap to avoid dissolving extraneous material from the cap lid and to avoid loss of solvent. Exactly 400 ml. of a mixed solvent (hexane and acetone, 3:1 by volume) are added to the sample. The sample and solvent are blended for 10 minutes, allowed to stand for two minutes, and then blended again for one minute. Up to 100 g. of anhydrous sodium sulphate may be added to the blender to facilitate the extraction. The blended sample is poured through a long-stem funnel containing a glass wool plug into a 500-ml. separatory funnel containing 250 ml. of water and 25 ml. of a saturated sodium sulfate solution. The funnel is shaken vigorously for one minute and then allow to stand until the aqueous and organic layers separate. The aqueous (lower) phase is discarded and the remaining hexane phase is filtered through a one-inch pad of anhydrous sodium sulfate and collected in a 16-oz. bottle.

γ) Alumina column cleanup. — A 30-ml. aliquot (equivalent to 10 g. of crop) of the hexane extract is transferred to a 250-ml. Erlenmeyer flask equipped with a ground glass joint) and brought to dryness using a flash evaporator. The sample is now ready for column cleanup.

A dry packed column is prepared by adding 25 g. of alumina basic, activity V to a column. The column is then tapped gently to eliminate channeling and to achieve uniform packing of the alumina. A glass wool plug is placed on top of the alumina. The sample residue is dissolved in two ml. of benzene. This solution is transferred to the column and allowed to penetrate into the alumina. The flask is washed with five ml. of *n*-hexane which is transferred to the column and allowed to penetrate as before. This operation is repeated with five ml. of *n*-hexane. A 250-ml. Erlenmeyer flask

is used as a receiver. When the last five ml. of wash *n*-hexane has just run into the column surface, 90 ml. of *n*-hexane is added and allowed to pass through the column. When the *n*-hexane has just run into the surface of the column, a clean 250-ml. flask is placed as receiver and 100 ml. of 1:1 hexane-benzene is added to the column and the elution is continued. The whole 100 ml. of eluant solution is then collected. The eluate is evaporated to dryness using a flash evaporator. The residue is transferred with hexane to a two-dram vial. The hexane is evaporated to dryness in a hot-water bath with the aid of a gentle stream of dry air. A volume of 0.2 to 0.5 ml. of benzene is added to the residue, the vial is shaken to ensure complete solution of the residue, and is stoppered. The sample is now ready for gas chromatography.

Ten percent benzene in *n*-hexane has been used as the first eluant for some samples containing large amounts of interfering material. The acaricide is not eluted by this solvent mixture.

δ) Gas chromatography. —

Injector temperature — 225 °C.
Column temperature — 200 °C.
Transfer tube temperature — 250 °C.
Furnace temperature — 875 °C.
Carrier — nitrogen, 110 ml./minute
O_2 flow — 50 ml./minute
N_2 purge — 20 ml./minute
Attenuation — 200 ohms
Titration cells — T-300-s (chloride specific)
Minimum detection limit — 10 ng.
Injection volume — 2 to 8 μl.
Chart speed — 0.5 inch/minute
Retention time — chlorobenzilate 3.3 minutes

The gas chromatograph response is standardized by injecting known amounts of analytical standard. A stock solution is prepared by dissolving 100 mg. of standard in 100 ml. of benzene from which an aliquot is diluted until a final concentration of 10 ng./μl. is reached. Volumes of two to eight μl. (equivalent to 20 to 80 ng.) are injected. Peak areas are determined by triangulation and standard curves are constructed by plotting ng. of acaricide standard injected against peak areas.

Recoveries of acaricide standards added to the check samples before extraction and carried through the whole analytical procedure are determined. The amount of acaricide recovered (ng.) is read from the standard curve from the peak areas obtained from the fortified samples. The p.p.m. values are then calculated by dividing the ng. of acaricide by the weight (mg.) of the crop equivalent injected. The percent recovery is then calculated based on the theoretical value added.

For unknown samples, the analyses and calculations are done as described above. In addition, corrections are made for the percent recovery obtained from the recovery studies.

5. Special procedure for oily samples. — Samples such as nuts, cottonseed, and citrus peel require separation of the oil before column chromatography. The hexane extract is evaporated on a steam bath to a volume of 50 ml. It is then transferred to a 250-ml. separatory funnel and the beaker is washed twice with 25 ml. portions of hexane. The acaricide is extracted from the hexane solution using two 25-ml. portions of acetonitrile. The combined acetonitrile extract is then washed with 50 ml. of hexane to remove traces of oil carried along. The acetonitrile containing the acaricide is transferred quantitatively into a 100-ml. round-bottom flask equipped with a 24/40 joint.

The acetonitrile is completely evaporated under reduced pressure (water aspirator) using a flash evaporator with the water bath at 50 °C. The sample is now ready for chromatographic cleanup, as described previously.

6. Samples of unknown history. — Practically all of the samples analyzed in our laboratory are samples whose history of treatment with pesticides is known. Some observations can, however, be made in regard to specificity of the methods described.

The chloride sensitive detector eliminates interference from non-halogenated compounds.

Interference from DDT and DDD (TDE) is eliminated by the cleanup procedure. These materials are eluted in the hexane fraction when using the alumina column and so are removed before gas chromatography. The DDT and DDD (TDE) content could be determined independently if so desired.

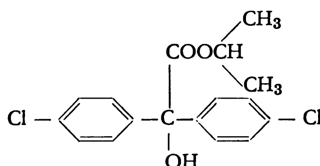
Specificity is also given by the elution times. Chlorobenzilate and chloropropylate peaks are resolved by the gas chromatographic column described above. Conversely, if separation is not required, these acaricides have been gas chromatographed successfully using the following liquid phases: GS-Nitrile XE-60, SE-30, and QF-1.

IX. Properties and characteristics of chloropropylate

Chemical names: isopropyl 4,4′-dichlorobenzilate
isopropyl-2-hydroxy-2,2-di(*p*-chlorophenyl) acetate (IUPAC)

Empirical formula: $C_{17}H_{16}Cl_2O_3$

Structural formula:



Molecular weight: 339.21

Physical properties:

The compound is a white powder with a melting point of 73° to 75° C. and a boiling point of 148° to 150° C. at 0.5 mm. of Hg. The vapour pressure is 8×10^{-4} mm. of Hg at 20° C. The technical product contains approximately 90 percent of the above compound. Its solubility in water is less than 0.001 percent = 10 p.p.m. at 20° C.; it is readily soluble in most organic solvents. Chloropropylate is stable in a neutral medium, but less stable under alkaline and acid conditions.

X. Toxicology of chloropropylate

a) Acute toxicity

The results of acute oral, dermal, and inhalation toxicity studies are shown in Table XV.

b) Subchronic and chronic toxicity

1. Short-term studies (oral administration). —

α) Rat. — Chloropropylate active ingredient, suspended in gum arabic, was administered by stomach tube six times a week for four weeks to three groups of rats each group consisting of five males and five females, at dosage levels of 50, 250, and 500 mg./kg./day. Based on the criteria chosen for the evaluation of the results (rate of survival, body-weight changes, symptoms, autopsy, and microscopic examination of tissues), no toxic effect whatsoever was noticed in the treated animals (*J. R. Geigy S. A.* 1962 c).

In a three-month feeding study, five groups of 21 male and 21 female rats each received food containing zero, 0.01, 0.1, 0.5, and 2.5 percent of chloropropylate active ingredient, equivalent to zero, 100, 1,000, 5,000, and 25,000 p.p.m. of chloropropylate. As judged by changes in body weight and food intake (recorded weekly), general behaviour, survival, gross pathology, and histopathological examination, there were no significant differences between the test groups and the control group with regard to food intake, with the exception of a reduction observed in the 25,000 p.p.m. group. The development of the growth curves was normal in both 100 p.p.m. groups and in the 1,000 p.p.m. group of females, while the 1,000 p.p.m. of males showed a slight, probably insignificant depression of body weight gain. At the end of the test, the animals of the 5,000 p.p.m. group had a body weight of 81 to 82 percent of the untreated check group, the corresponding figure being 32 to 38 percent for the 25,000 p.p.m. group. No deaths occurred in the 100 and 1,000 p.p.m. groups. In the 5,000 p.p.m. group the mortality was 29 percent, and in the 25,000 p.p.m. group the corresponding figure was 71 percent, without a significant difference between males and females. Autopsies revealed no gross pathological changes that could be attributed to the administration of chloropropylate. The histopathological examination gave the following findings: in the 100 p.p.m.

Table XV. *Toxicity evaluations of chloropropylate*

Animal	Route	Formulation or solvent	LD_{50} (mg./kg. body weight) [a, b]	References
Mouse	oral	suspension in gum arabic	$> 5,000$	*J. R. Geigy S. A.* (1962 a)
Rat	oral	suspension in gum arabic	$> 5,000$	*J. R. Geigy S. A.* (1962 b)
Rat	oral	40% wettable powder	$> 13,840$	*Ind. Bio-Test Lab.* (1965 e)
Rat	oral	25% emulsifiable sol.	1,250	*Ind. Bio-Test Lab.* (1965 d)
Fowl	oral	25% emulsifiable sol.	2,500	SANDERSON (1963)
Rabbit	dermal	40% wettable powder	$> 4,080$	*Ind. Bio-Test Lab.* (1965 e)
Rabbit	dermal	25% emulsifiable sol.	$> 2,550$	*Ind. Bio-Test Lab.* (1965 d)
Rat	inhalation	dust (40% WP; undiluted)	LC_{50} [c] > 3.8 mg./l. air	*Ind. Bio-Test Lab.* (1965 e)
Rat	inhalation	aerosol (40% WP; 5% aq. suspension)	LC_{50} [c] > 10.8 mg./l. air	*Ind. Bio-Test Lab.* (1965 e)
Rat	inhalation	aerosol (25% ES; undiluted)	LC_{50} [c] < 47.0 mg./l. air	*Ind. Bio-Test Lab.* (1965 d)
Rat	inhalation	aerosol (25% ES; 30% aq. sol.)	LC_{50} [c] ~ 46.2 mg./l. air	*Ind. Bio-Test Lab.* (1965 d)
Rabbit	eye irritation test	40 W; 100 mg. of undiluted test material instilled into the conjunctival sac	mildly irritating	*Ind. Bio-Test Lab.* (1965 e)
Rabbit	eye irritation test	25 E; 0.1 ml. of undiluted test material instilled into the conjunctival sac	extremely irritating	*Ind. Bio-Test Lab.* (1965 d)

[a] All values are expressed in terms of active ingredient.

[b] The symbol $>$ means that the LD_{50} value is higher than the quoted figure, *i. e.*, the highest tested.

[c] The LC_{50} values are expressed in terms of the formulations used based on a four-hour exposure.

group no changes were observed, but in the 1,000 p.p.m. group the livers regularly showed an increase of deposit of neutral fat in the centro- to medio-lobular epithelium; the nuclei of the liver cells were, however, unchanged. In two out of 21 males a testicular atrophy with various stages of maturation inhibition of spermatids and spermatocytes was observed. The ovaries were unchanged. In the 5,000 and 25,000 p.p.m. groups these pathological changes were more pronounced with a higher incidence of testicular atrophy (*J. R. Geigy S. A.* 1963).

β) Dog. — For three months Chloropropylate 50W was fed to four groups of three male and three female beagle dogs at dietary levels of zero, 100, 500, and 3,000 p.p.m., expressed in terms of active ingredient. Upon completion of the study, all dogs were sacrificed and gross autopsies performed. As judged by the criteria chosen for the evaluation of the effect (appearance, body weight gains, appetite, survival, hematological and biochemical studies, organ weights and organ/body weight ratios, and gross and microscopic pathology), chloropropylate was well tolerated by the dogs at all dosages, and there were no clinical signs of effect at any level. One male dog of the 3,000 p.p.m. group lost 0.8 kg. of body weight; the other dogs essentially maintained their body weights. Based on the results of hematological and biochemical studies and urine analyses, organ weights, and gross and microscopic findings, the test dogs were comparable with the control dogs (*Hazleton Laboratories* 1965 b).

2. Long-term studies (oral administration). —

α) Rat. — In a two-year chronic toxicity study, three groups of rats consisting of 30 males and 30 females each were fed Chloropropylate 50W at dietary levels of zero, 40, and 125 p.p.m., expressed in terms of active ingredient. From the parameters chosen (general appearance, behaviour, survival, body weights, and histopatholgy including frequency of neoplasms) it is concluded that the dose of 40 p.p.m. is a no-effect level. No adverse effects were noted in the 125 p.p.m. group with the possible exception of a slight decrease in mean absolute and relative prostate weights in the males as compared with those of the control group. However, no greater frequency of prostate changes were determined by histopathological observations in the test groups than in the control group (*Woodard Research Corp.* 1966 f).

β) Dog. — In a two-year feeding study Chloropropylate 40W was administered to groups of three male and three female beagle dogs at dietary levels of zero, 100, 500, and 3,000 p.p.m., expressed in terms of active ingredient. Because of adverse reactions and mortality occurring shortly after the initiation of the study, the highest dose level was altered as follows: 3,000 p.p.m. administered during days one to 103, then reduced to 2,000 p.p.m. (days 104 to 116), off-test days 117 to 197, again 2,000 p.p.m. during days 198 to 795. Three further dogs from a 90-day subacute oral toxicity study at 3,000 p.p.m. were added to the 2,000 p.p.m. group on day 330 to fill out this group. As judged from the criteria chosen (body weight,

food consumption, reactions, mortality, hematology, clinical chemistry, organ weights, gross pathology, and histological examination), chloropropylate caused no significant adverse effects at the 100 and 500 p.p.m. levels, with the exception of a slight depression of growth rate in two out of three female dogs of the 500 p.p.m. group, which, however, does not seem to be significant.

Among the dogs of the 3,000 to 2,000 p.p.m. level, poor weight gains (1.4 kg. as compared to 4.5 kg. in the control group), mortality (three out of six), elevation of serum alkaline phosphatase, and an increase in absolute liver weights and liver weight/body weight ratios occurred. Since no gross or microscopic abnormalities were noted among the livers of the test animals, the increase in size of the livers was obviously caused by physiological response to the stress of the ingestion of high levels of the compound. No pathological changes, either gross or microscopic, were observed in any of the test animals, which could be attributed to the two-year administration of chloropropylate (*Industrial Bio-Test Laboratories* 1968).

3. Repeated dermal application. —

α) Rabbit. — In a 21-day subacute dermal toxicity study Chloropropylate 40W was applied as a 50 percent aqueous suspension to the skin of four test groups consisting of five male and five femal rabbits each. Two groups (intact and scarified skin) were treated at a dose level of 1,000 mg./kg./day, while the two other groups received 2,000 mg./kg./day (intact and scarified skin). Doses are expressed in terms of Chloropropylate 40W, and not in terms of the 50 percent aqueous suspension as prepared. The test material remained in contact with the skin for a period of seven hours/day, five days/week, for three weeks, or a total of 15 seven-hour applications. The results were as follows: four out of 20 animals in the two test groups of 1,000 mg./kg./day, and six out of 20 animals in the two test groups of 2,000 mg./kg./day died. Statistical analysis of total weight gain data obtained from surviving animals revealed no significant difference from those of the control group animals. A thickening of the skin at the application site was noted in all test group animals. With the exception of this thickening of the skin, no significant gross pathological and histopathological changes were noted in any of the tissues and organs examined (*Industrial Bio-Test Laboratories* 1966).

A similar 21-day subacute dermal toxicity study was carried out with Chloropropylate 25E. Skin applications of the undiluted test material were made to four groups (intact and scarified skin) of rabbits at dose levels of 1,000 and 2,000 mg./kg./day, respectively. The method used was the same as described above. The results were as follows: four out of 20 animals in the two groups of 1,000 mg./kg./day and three out of 20 animals in the two groups of 2,000 mg./kg./day died. Local skin reactions characterized by severe erythema and edema and slight adverse body weight effects were observed in the treated animals. With the exception of the skin at the application site of the treated animals, no gross pathological alterations were found

in the tissues and organs examined. The histological examination showed skin alterations, but no other pathological changes (*Industrial Bio-Test Laboratories* 1967 b).

It should be taken into consideration that a 50 percent aqueous suspension and an undiluted 25 percent emulsifiable concentrate, both at exaggerated levels, were used in these dermal toxicity studies. Under field conditions pest control operators are never subjected to this concentrated material nor to the severe conditions under which these studies were performed.

β) Man. — A repeated insult patch test was conducted in 50 human volunteers with technical chloropropylate tested as a one percent emulsion in water. Under the conditions of this test, none of the subjects reacted to any of the primary applications or to the challenge. Technical chloropropylate, as tested, proved to be neither a primary irritant nor a fatiguing agent. There was no evidence that the test material produced any sensitizing action (*Industrial Biology Laboratories* 1965).

c) Special studies

1. Reproduction in the rat. — In a three-generation reproduction study the feeding of Chloropropylate 50W at dietary levels of zero, 25, and 50 p.p.m., expressed in terms of active ingredient, to three groups of rats of both sexes (10 male and 20 female rats) had no adverse effect on the reproduction as judged by the criteria evaluated. Test and control groups of parent rats were comparable throughout the study in mean body weights, general appearance and behaviour, mortality, and reproductive capacity. The litters from the second matings were used to provide the new generations. Corresponding test and control litters of the three generations were comparable in number of litters/group, number of live births, physical condition, mean weights at birth and weaning, percent young alive at weaning, and gross autopsy observations. Examination of females of the F_1b and F_2b generations that had fewer than two litters for uterine implantation sites revealed no effect on fetal resorption related to the administration of chloropropylate. No malformations were observed in any of the offsprings. Body, heart, liver, and kidney weights of test and control weanlings of the F_3b litters were comparable. Histopathological observations on tissues of weanlings in each F_3b litter indicated a slightly higher frequency of commonly encountered hepatic cell vacuolation and mineralization in renal tubules in pups from treated females than in pups from control females. However, all of these appearances are such as are commonly encountered in normal, untreated weanling laboratory rats (*Woodard Research Corp.* 1966 g and 1967).

2. Effects on fish and wildlife. —

α) Wild birds. — Bobwhite quail (Colinus virginianus). Chloropropylate 40W was administered by incorporation into the diet to three groups of bobwhite quail each at dietary levels of 20,000, 10,000, and 5,000 p.p.m.

(equivalent to 8,000, 4,000, and 2,000 p.p.m. of active ingredient). In comparison, five groups of bobwhite quail, each group consisting of five animals, were fed 77.2 percent *p,p'*-DDT at dietary levels of 3,200, 1,800, 1,000, 560, and 320 p.p.m. The quail were maintained on diets containing the above-mentioned concentration of materials for seven days. In terms of active ingredient the LC_{50} was found to be 4,000 p.p.m. for chloropropylate and 1,273 p.p.m. for *p,p'*-DDT (*Woodard Research Corp. 1966* c).

Mallard ducks (Aras platyrhynchus). Nine groups, consisting of 10 mallard ducks each, were maintained for five days on diets containing zero, 20,000, 10,000, and 5,000 p.p.m. of Chloropropylate 40W (equivalent to 8,000, 4,000, and 2,000 p.p.m. of active ingredient) and 3,200, 1800, 1,000, 560, and 320 p.p.m. of 77.2 percent *p,p'*-DDT. Using mortality, food consumption, body weight, and gross necropsy findings as criteria for the evaluation of the effect, it was found that Chloropropylate 40W has an LC_{50} greater than 20,000 p.p.m., as compared to 1,400 p.p.m. for 77.2 percent *p,p'*-DDT. In terms of active ingredient the LC_{50} is $>$8,000 p.p.m. for chloropropylate and 1,081 p.p.m. for *p,p'*-DDT. Chloropropylate 40W at the highest dose level reduced food consumption and weight gain (*Woodard Research Corp.* 1966 d).

β) Fish. — Rainbow trout (Salmo gairdneri). Groups of five fish each were placed in glass jars containing 15 liters of water and exposed for 96 hours to concentrations of chloropropylate ranging from 0.18 to 1.8 p.p.m., expressed in terms of active ingredient, and to *p,p'*-DDT at concentrations of 0.0010 to 0.010 p.p.m. active ingredient. Mortality was recorded during each 24-hour period. After 96 hours of exposure, the LC_{50} was as follows: chloropropylate 0.45 p.p.m., and *p,p'*-DDT 0.0018 p.p.m. (*Woodard Research Corp.* 1966 c).

Bluegill sunfish (Lepomis macrochirus) and goldfish (Carrassius auratus). In similar fish toxicity tests as described above, the following LC_{50} values were determined for these two species, based on a 96-hour exposure (Table XVI).

Table XVI. *LC_{50} values for two species of fish*

Species	LC_{50} (p.p.m. active ingredient)	
	Chloropropylate	*p,p'*-DDT
Bluegill sunfish	0.66	0.0047
Goldfish	0.60	0.0070

These data show that chloropropylate is markedly less toxic than DDT to these two fish species (*Woodard Research Corp.* 1966 c).

Harlequin fish (Rasbora hetromorpha). Results obtained by the *Ministry of Agriculture* of the United Kingdom *(Freshwater Fisheries Laboratory)* indicate that the 24-hour median tolerance limit of harlequin fish for the 25 percent ES formulation of chloropropylate was 20 p.p.m. [*Chesterford Park Research Station,* N. Saffron Walden, Essex (1964)].

Oyster (Ostrea virginica). Oysters were exposed to technical chloropropylate and *p,p'*-DDT in flowing sea water aquaria for one week. Based on mortality and shell growth as criteria, it was found that a 50 percent reduction of shell growth was produced by 0.5 p.p.m. of chloropropylate and 0.24 p.p.m. of *p,p'*-DDT, respectively, both values being expressed in terms of active ingredient. Concentrations of 1.0 or 0.5 p.p.m. of chloropropylate active ingredient caused no mortality, while *p,p'*-DDT at 0.5 p.p.m. caused a 35 percent mortality (*Woodard Research Corp.* 1966 e).

d) Acceptable daily intake

On the basis of the present toxicological data, the 1968 Joint FAO/WHO Meeting on Pesticide Residues established as temporary acceptable daily intake for man a value of 0.01 mg./kg. body weight [*World Health Organization*, Techn. Report Series No. 417 (1969)]. Therefore, a 60-kg. person would tolerate a daily dosage of 0.60 mg. of chloropropylate without appreciable risk.

XI. Metabolism of chloropropylate in animals

Metabolic studies following oral administration of chloropropylate have been performed in dogs and rats.

In the dog study, technical chloropropylate was administered by capsules, daily for five days, to two groups of mongrel dogs, each group consisting of one male and one female, at dosage levels of 12.8 and 64.1 mg./kg., respectively. Daily urine and total fecal specimens pooled were analyzed. The males excreted 5.20 and 33.16 percent respectively, of the total doses (12.8 and 64.1 mg./kg.) in the urine; the corresponding figures for the females were 3.17 and 33.69 percent, respectively. In the feces, 6.42 and 3.37 percent, respectively, were found in the males at the low and the high doses, and 16.91 and 7.32 percent, respectively, in the females. At the end of 10 days, each animal including the controls was sacrificed and tissues (blood, brain, kidney, fat, liver, and muscle) analyzed for residues. No stored chloropropylate was found in any of these tissues. As was already shown for chlorobenzilate, also chloropropylate hydrolyzes at the ester linkage resulting in the formation of dichlorobenzilic acid. This compound is determined by the Schechter-Haller procedure (*Hazleton Laboratories* 1964). However, since the colorimetric method can determine other similar compounds, thin-layer chromatography was used to supplement the colorimetric analyses by a specific method for the detection of dichlorobenzilic acid. Ether extract of urine from the dogs treated with chloropropylate contained a material which, when chromatogrammed, corresponded to dichlorobenzilic acid (MATTSON *et al.* 1965). The results of the TLC analysis are shown in Table XVII.

In a balance study, radioactive C^{14}-chloropropylate was administered by oral intubation to two male and two female rats at a dosage of about 1.6 mg./kg. The excretion of radioactivity in urine, feces, and exhaled CO_2

Table XVII. *TLC analysis of urine from treated dogs*

Sample	Material administered	Days after treatment	Dichlorobenzilic acid
Control urine	—	—	absent
Standard dichlorobenzilic acid in control urine	—		present
Urine from dog given 64.1 mg. of chloropropylate/kg./day	chloropropylate	2	present
	chloropropylate	3	present
	chloropropylate	4	present
	chloropropylate	5	present

was studied during a 120-hour period. The rats were then sacrificed, and various tissues and organs were analyzed for radioactive residues. The study clearly showed that the excretion pattern for male and female rats was different. The majority of the radioactivity was excreted in the feces (75 percent by males and 49 percent by females). The urine of females contained 31 percent of the radioactivity, whereas in the urine of males only six percent was found. No significant radioactive CO_2 was expired by the rats during the study. Most of the radioactivity was eliminated in the first 48 hours. Only small amounts of radioactivity (0.9 percent in males and one percent in females) remained in the tissues 120 hours after administration. Most of this radioactivity was found in liver and fat (CASSIDY *et al.* 1968).

XII. Performance of chloropropylate

a) Mode of action

1. Acaricidal action. — Chloropropylate controls *summer eggs* and all *postembryonic stages* of most plant injuring mites (GASSER and GROB 1963). The following Table XVIII presents the results of a laboratory experiment. Eggs, dormant, and mobile stages of a normally susceptible *Tetranychus urticae* strain were treated with chloropropylate and chlorobenzilate at various concentrations.

Table XVIII. *Concentration vs. efficacy against T. urticae*

Concentration and compound	Percentage of stages [a] destroyed after 6 days								
	M	D	E	M	D	E	M	D	E
Conc. in % a.i.	0.01			0.005			0.0001		
Chloropropylate	100	100	100	100	100	62	86	0	0
Chlorobenzilate	100	100	100	100	100	87	95	0	0

[a] M = mobile stages (larvae, protonymphs, deutonymphs, adults).
D = dormant stages (nympho-, deuto- and teleochrysalides).
E = eggs.

Mobile and dormant stages can be 100 percent killed with five g. a.i./100 l.; for complete control of eggs under laboratory conditions at least 10 g. a.i./100 l. are necessary. Under laboratory conditions also all stages of a *T. urticae* strain resistant to diazinon, phenkapton, parathion, and demeton can be controlled (*J. R. Geigy S. A.* 1964).

As a *contact acaricide*, chloropropylate must be carefully applied on the entire surface of the plants. Only those mites that come into direct contact with the active ingredient are killed. Therefore, the product is less suitable for treatments of strawberries, since the mites are well protected by the leaves near the ground (SCHAEFERS 1965).

2. Knockdown and residual control. — The data of five chloropropylate treatments (at 25 g. a.i./100 l. each) carried out in the Rhône Valley, Switzerland, are presented in Table XIX. The data given in this table display the

Table XIX. *Knockdown and residual control against mites*

Crop and mite strain	Control (%)	Days assessed after treatment
Apple *(P. ulmi)*	96	1
	99	10
	95	15
Grape *(P. ulmi)*	95	3
	99	24
	96	34

knockdown effect of chloropropylate; already one to three days after treatment more than 95 percent of the mites are killed. This good effect lasts for two weeks and after many treatments for four weeks (CABANE 1967).

3. Effect on predators and bees. — Chloroporpylate is a selective acaricide which does not affect the useful insects (such as larvae of *Neuroptera* and *Diptera*, hyperparasitic *Hymenoptera*, and predatory *Coccinellidae*). Predaceous mites which kept down the spider mite population are hardly impaired by chloropropylate treatments.

Chlorpropylate applications are harmless to bees at any time. According to JOHANSEN (1966), the toxicity of chloropropylate to bees in the field is low or not existing at all. According to bee toxicity studies by BERAN [2], the LD_{50} *per os* is 160 μg. of chloropropylate 25 ES/bee. The rating index for the toxicity to bees I_s is 0.02, a figure that means complete innocuity to bees, all the more so since products with a danger index of up to one are already innocuous to bees in practice (BERAN and NEURURER 1956). The experiments were carried out on Phacelia in the glasshouse and in the field, showing that in fact no bee losses occurred after blossom sprays with a 25 percent emulsifiable solution of chloropropylate.

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[2] We are very much obliged to Prof. Dr. F. BERAN for the trial results.

b) Fields of application and recommendations

1. Field trial experiences. — In 1961, when strains of the European spider mite *(Panonychus ulmi)* resistant to organic phosphates were found in various orchards and vineyards in the Swiss Rhône Valley, the two acaricides chlorobenzilate and chloropropylate were compared under these conditions. These trials showed in fact that both products had an equally good initial effect against susceptible and resistant mites in orchards and vineyards, but that chloropropylate provided longer control; moreover, chloropropylate is better tolerated by stone fruit and can, therefore, be used for treatments in peach, plum, and apricot growing (GASSER and GROB 1963).

After many years of testing, such recommendations for chloropropylate dosages were developed as would achieve a satisfactory control of mite pests. The following Table XX gives a list of mites arranged after crops, which can be satisfactorily controlled with chloropropylate at the dosage rates indicated.

Table XX. *Mites and recommended dosages for control*

Culture	Mites	Recommended dosages
Pip fruits apples, pears	*Panonychus ulmi* K. *Tetranychus urticae* K. *Bryobia praetiosa* K. *Bryobia rubrioculus* Sch. *Epitrimerus piri* N.	1—2 pt. of 25 EC/100 U.S. gal. =30—60 g. of a.i./100 l. (for Pip fruits through Ornamentals)
Stone fruits peaches, apricots, cherries, plums	*Tetranychidae* *Aculus cornutus* B. *Bryobia praetiosa* K.	
Citrus oranges, lemons, grapefruit	*Tetranychidae* *Phyllocoptruta oleivora* A. *Aculus pelekassi* K.	
Grapes	*Panonychus ulmi* K. *Tetranychus urticae* K. *Eotetranychus carpini vitis* Oud.	
Tea	*Oligonychus coffeae* N. *Eriophyes theae* W. *Brevipalpus phoenicis* G.	
Ornamentals carnations, hydrangeas, roses, Linden trees	*Tetranychus urticae* K. *Eotetranychus tiliarium* Herm.	
Field crops and vegetables cotton, soybeans, sugar beets, cucumbers, beans, tomatoes, cantaloupes	*Tetranychus urticae* K. *Tetranychus cinnabarinus* B.	1.25—8 lbs. of 25 EC/acre =0.375—2.2 kg. of a.i./ha.

Chloropropylate is a specific *acaricide* that is mainly used in *fruit growing*. Very good results were reported from the fruit growing areas of Michigan (U.S.A.), where the product was used for pre-blossom and summer sprays against *Panonychus ulmi* and *Tetranychus urticae* (MATLICK 1967). According to investigations carried out by the *Michigan Agricultural Experimental Station, Michigan State University,* chloropropylate can be applied by means of ultra-low-volume sprays (0.5 lb. a.i./960 ml./acre) with especially equipped air-blast sprayers. Treatments with 25 g. a.i./100 l. provide satisfactory three weeks' control of *Bryobia rubrioculus,* the vicarious form of *B. praetiosa* in Europe (CHABOUSSOU 1956). The product can be successfully applied against this mite in peaches, without injuring the crop (CHABOUSSOU 1956). Since chloropropylate is a contact acaricide, spraying must be very thorough, so that all parts of the trees are covered.

In viticulture chloropropylate at a rate of 30 g. a.i./100 l. controls the most important *Tetranychidae* (RAMBIER 1958). Today the well-introduced product chlorobenzilate is preferred in *citrus,* although chloropropylate is effective against citrus mites as well. In a comparative test, chloropropylate was even found to be the most effective material among 63 substances against the two citrus rust mites *Phyllocoptruta oleivora* and *Aculus pelekassi.* Trials carried out at the *Tocklai Experimental Station* (Annual Scientific Report 1964) in Assam, India, showed that chloropropylate was a good product for the control of the tea red spider mite, *Oligonychus coffeae,* and the pink mite, *Eriophyes theae,* in *tea.* One month after treatment the product still displayed 98 percent effect against *O. coffeae.* Chloropropylate does not impair the flavour of tea. In *ornamental plants, field, and vegetable crops* that are most frequently attacked by spider mites, chloropropylate is a promising product, even against mites resistant to phosphates (FURR and DAVIS 1969).

2. Directions for applications. — In general, chloropropylate treatments should be carried out early at the beginning of vegetation and when mites appear first. Experience has shown that in fruit growing the best time of application is immediately before blossoming (pink sprays) when the first larvae hatch. Generally, one application made at this time and with good coverage controlled mites until mid-summer. In some instances season-long mite control was obtained and summer miticide applications were not necessary. For preblossom sprays 45 g. a.i./100 l. in sufficient spray mixture should be used. Also postblossom sprays are successful, if carried out at the onset of infestation. In areas where a high population existed, the best control was achieved by making two applications 10 to 12 days apart. The rate used for summer sprays is 60 g. a.i./100 l. Decisive for the success of a chloropropylate treatment is not only the dosage level, but also the spray volume, the latter being dependent on the spraying equipment. There must be sufficient spray volume to achieve thorough wetting of all plant parts. According to CABANE (1967), chloropropylate applications at 25 g. a.i./100 l. in 2,000 l. of spray volume are more effective than 50 g. a.i./100 l. in 1,000 l. of water. GASSER and GROB (1963) recommend 3,000 to 4,000 l. of spray

mix/ha. In citrus, the spray volume should not exceed 9,000 to 10,000 l./ha. If necessary, treatments can be repeated.

c) *Phytotoxicity*

Pome fruit is highly tolerant to chloropropylate. Even after several treatments with 45 to 60 g. a.i./100 l. at short intervals, no damage was reported with the pear varieties Bartlett, Seckel, D'Anjou, and Williams and the apple varieties Delicious, Jonathan, and McIntosh that are susceptible to chlorobenzilate under certain conditions.

Chloropropylate is by far better tolerated by *stone fruit* than is chlorobenzilate. Two chlorobenzilate treatments (at 25 g. a.i./100 l. each, at intervals of 12 days) caused such excessive peach leaf drop that the damage remained for the whole vegetation period. On the trees that had been sprayed with chloropropylate only slight leaf fall occurred four weeks after treatment, but already after a short time no difference between treated and untreated trees was observed. Plums and prunes that have a varietal susceptibility to chlorobenzilate reacted much less or not at all to chloropropylate (*J. R. Geigy S. A.* 1955). According to investigations carried out by various American experimental stations, peaches (variety Elberta) and plums (variety Fellenberger) proved to be tolerant to chloropropylate. Chloropropylate, however, should not be used in combination with oils in stone fruit, as injury may occur.

Symptoms of phytotoxicity, as the occurred with some *grape vine varieties* after preblossom sprays with chlorobenzilate, were not observed after chloropropylate applications.

XIII. Trademarks and formulations of chloropropylate

Chloropropylate was developed by *J. R. Geigy S. A.*, Basel, Switzerland, and introduced under the registered trademarks shown in Table XXI.

Table XXI. *Trademarks and formulations of chloropropylate*

Commercial designation	Technical chloropropylate in (%)	Formulation
ROSPIN 20	20	emulsifiable solution
CHLOROMITE	24.5	emulsifiable solution
ROSPIN/ROSPAN 25	25	emulsifiable solution
ACARALATE 2E	25.2	emulsifiable solution
GESAKAR 25	25	emulsifiable solution

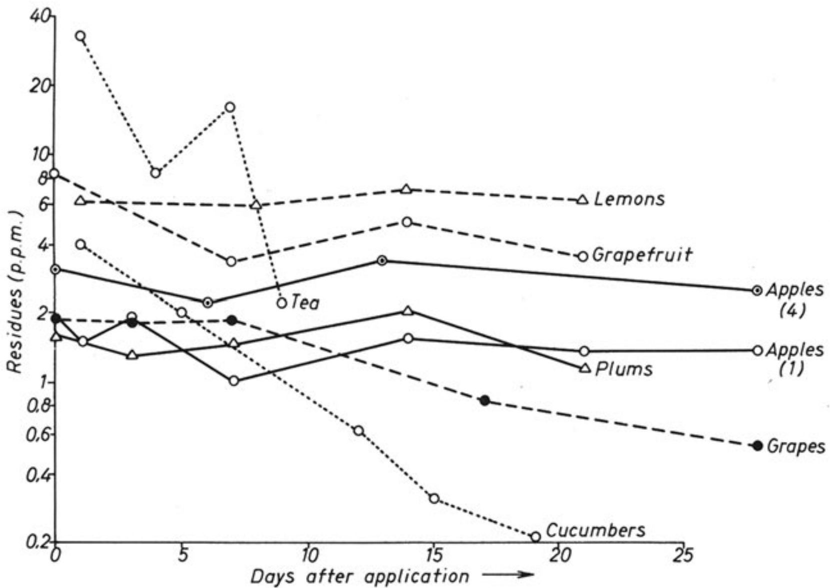

Fig. 2. Dissipation curves of chloropropylate: apples (1) = one spray of 50 g. a.i./100 l., apples (4) = four sprays of 60 g. a.i./100 l., plums = two sprays of 60 g. a.i./100 l., grapefruit = one spray of 60 g. a.i./100 l., lemons = one spray of 45 g. a.i./100 l., grapes = two sprays of 60 g. a.i./100 l., tea = one spray of 50 g. a.i./100 l., and cucumbers = one spray of 25 g. a.i./100 l.

XIV. Residues of chloropropylate

a) Residues from supervised trials

Pome fruit, stone fruit, citrus, tea, and vegetables, treated at varying rates, were analysed for chloropropylate residues. Table XXII presents a number of analyses from the U.S.A., Switzerland, and the U. K. Most of these data had been obtained by a gas chromatographic method. Some of these results are presented in Figure 2 graphically showing the dissipation curves of chloropropylate.

On *apples* and *pears* the initial residue level (deposits immediately after last treatment) is hardly above five p.p.m.; 90 percent of the active ingredient are found in the peel and up to 10 percent in the meat of the fruit. The highest residue found in apple meat one day after a chloropropylate application at a high rate (1.12 kg.a.i./ha.) was 0.48 p.p.m. (see Table XXII). Furthermore, it should be mentioned that the residues found on apples treated four times (total dosage applied 240 g. a.i./100 l.) are only one p.p.m. higher than on apples treated only once at 50 g. a.i./100 l. The velocity of

dissipation is the very same after both applications. The chloropropylate residue level was reduced by about 0.7 p.p.m. after four weeks (see Fig. 2).

The initial residues on *stone fruit* (nectarines, prunes, plums) are below three p.p.m. after treatments at the recommended dosage rates (60 g. a.i./100 l.) and are generally reduced to one p.p.m. after three weeks.

With *citrus fruits* (grapefruit, lemons, oranges) the chloropropylate residues are found exclusively in the peel, which is as an even more efficient barrier against the penetration of the acaricide into the pulp than with pome fruit. In contrast to the peel of oranges, the peel of lemons and grapefruit contains high residues (between six and eight p.p.m.) which dissipate only very slowly. The temporary tolerance of three p.p.m. proposed for citrus fruit (whole-fruit basis) by FAO/WHO is certainly not exceeded if the present values determined separately for peel and pulp are put in relation to the whole fruit.

The initial residue level on *vine grapes* is near two p.p.m.; these residues are only slowly reduced and come to one to two p.p.m. after four weeks, depending on the dosage applied. Dried *tea* leaves show high residues one day after treatment, yet chloropropylate disappears from the dried leaves fairly rapidly, for after 10 days residues of 0.2 and 2.3 p.p.m., respectively, can be found. The acaricide is more rapidly degraded by fermentation than by mere drying. Made tea (after ferment action) has only 2/3 or so of the residues found on dried leaves. After brewing, 19.7 percent of the residue was found in the brew (corresponding to 0.01 p.p.m., see Table XXII). The remainder of the residue was in the wet leaves, based upon the residue found in the dried tea leaves. This recovery showed that no significant amount of degradation of the chloropropylate had occurred during brewing of the tea.

On *cucurbits* (cucumbers, melons) and *tomatoes* the residue level is low. The growth dilution, *i. e.*, this seeming dissipation of spray residues, is greatly due to the rather rapid growth of these fruits.

b) Behaviour and fate of the residues

Figure 2 shows that the residues on pome, stone, and citrus fruits degrade only very slowly. Investigations carried out by Hassan and Knowles (1969) inform of the fate of chloropropylate applied on plant tissue. Residue analyses of soybean leaves treated with ^{14}C-labelled chloropropylate showed that the substance is rapidly taken up by the leaf tissue and that only a small part remains on the surface [3]. The chloropropylate in the leaf tissue is not metabolized. Also, according to investigations by Murphy (1966), chloropropylate remains chemically unchanged in plant tissue (apple peel

[3] Four days after application 34 percent of chloropropylate is already in the leaf homogenate and 27.7 percent in the leaf rinse after washing.

Table XXII. *Chloropropylate residues in various crops*

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p.p.m.) and plant parts analyzed
								fruits
Apples	Switzer-land (1964)	G.B.	0.1% of 25 ES 25 g. a.i./100 l.	—	1	0	GC	1.2
						1		1.8
						3		1.0
						7		0.8
						14		1.3
						21		0.9
						28		0.7
						35		0.7
						42		0.7
		G.B.	0.2% of 25 EC 50 g. a.i./100 l.	—	1	0	GC	2.0
						1		1.5
						3		1.9
						7		1.0
						14		1.5
						21		1.3
						28		1.3
						35		0.7
						42		0.6
Apples Worcester Pearmain	U.K. (1963)	CH.E.	0.1% of 25 EC	30. V./19. and 26. VII.	3	28	Col.	2.17
			0.2% of 25 EC	27. VIII.	1	1	Col.	0.76
			0.2% of 25 EC	27. VIII.	1	27	Col.	0.92
Apples Cortland	U.S.A. Wisconsin (1966)	G.A.	2 lb. of 25 EC 60 g. a.i./100 l.		2	77	GC	< 0.1
						126		< 0.1

Apples	U.S.A.	G.A.	2 lb. of 2 E	sprayed to run-off	4	0	GC		3.1
Red Delicious	Oregon		60 g. a.i./100 l.	29. VI./13. VII.		6			2.2
	(1967)			1. VIII./16. VIII.		13			3.3
						30			2.3
						42			1.3
Apples	U.S.A.	G.A.	1.5 lb. of 2 E	21. VI./5. VII.	2	0	GC		5.7
Red Delicious	N. York		45 g. a.i./100 l.	spray mixture: 300 gal./acre		7			3.0
	(1969)					14			2.6
						21			1.7
						28			1.4
								peel	*meat*
Apples	U.S.A.	G.A.	1 lb. of 25 EC	—	1	1	GC	0.62	0.07
	Calif.		0.28 kg. a.i./ha.			7		0.47	< 0.04
	(1964)					14		0.40	< 0.04
						40		0.30	< 0.04
		G.A.	2 lb. of 25 EC	—	1	1	GC	2.2	0.22
			0.56 kg. a.i./ha.			7		1.3	< 0.04
						14		0.88	< 0.04
						40		0.63	< 0.04
		G.A.	4 lb. of 25 EC	—	1	1	GC	4.6	0.48
			1.12 kg. a.i./ha.			7		3.0	< 0.04
						14		3.4	< 0.04
						40		1.7	0.06

[a] References: GB = *J. R. Geigy A.G.*, Basel: Residue reports, unpublished.
GA = *Geigy Chemical Corp.*, Ardsley, N.Y.: Residue reports, unpublished.
Ch.E. = *Chesterford Park Research Station*, Saffron Walden, Essex, England: Residues of chloropropylate in sprayed apples, cucumbers. Unpublished report from C. A. Benfield and D. Richardson, *Fisons Pest Control Limited*, Report CP/63/ANAL/20.

[b] Formulation: EC = emulsifiable concentrate, E = emulsifiable concentrate, and WP = wettable powder.

[c] Analytical method: GC = gas chromatography and Col. = colorimetry.

[d] Treated leaves (461 kg.) were mixed with untreated leaves (1,845 kg.) to afford a 1 : 4 mixture.

Table XXII (continued)

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p.p.m.) and plant parts analyzed		
								peel	*meat*	*whole fruit*
Apples	U.S.A. Virginia (1964)	G.A.	4 lb. of 25 EC 1.12 kg. a.i./ha.	—	1	1	GC	—	—	3.2
						7		—	—	0.06
						14		—	—	2.1
						21		—	—	1.5
						40		—	—	2.1
		G.A.	8 lb. of 25 EC 2.24 kg. a.i./ha.	—	1	1	GC	—	—	4.6
						7		—	—	4.1
						14		—	—	4.5
						21		—	—	3.6
						40		12.0	0.1	3.5
								peel	*meat*	*whole fruit*
Pears	U.S.A. Calif. (1964)	G.A.	1 lb. of 25 EC 0.28 kg. a.i./ha.	—	1	1	GC	1.6	< 0.04	—
						1		—	—	0.77
						7		—	—	0.14
						14		—	—	0.14
						40		0.29	< 0.04	—
		G.A.	2 lb. of 25 EC 0.56 a.i./ha.	—	1	1		3.7	0.04	—
						1		—	—	0.21
						7		—	—	0.70
						14		—	—	0.38
						40		2.5	< 0.04	—

		G.A.	4 lb. of 25 EC 1.12 kg. a.i./ha.	—	1	1		7.1	0.19	—
						1		—	—	—
						7		—	—	0.63
						14		—	—	0.48
						40		2.2	< 0.04	—
									berries	
Blueberry Murphy	U.S.A. N. Carol. (1966)	G.A.	2 lb. of 25 EC 0.56 a.i./ha.	24. V./3. VI.	2	0	GC		15.0	
						0			24.0	
						3			5.1	
						3			8.3	
						7			6.2	
						7			6.0	
						13			2.1	
						13			0.95	
						21			2.9	
						21			1.3	
		G.A.	4 lb. of 25 EC 1.12 kg. a.i./ha.	As above	2	0	GC		44.0	
						0			40.0	
						3			20.0	
						3			20.0	
						7			14.0	
						7			8.3	
						13			9.0	
						13			8.4	
						21			3.9	
						21			1.3	
									fruit	
Nectarines Stanwick	U.S.A. Calif. (1966)	G.A.	2 lb. of 25 EC 60 g. a.i./100 l.	16. VI. /22. VII. growth stage of the fruits: mature spray mixture: 300—500 gal./acre	2	0	GC		1.9	
						0			2.0	
						3			1.0	
						3			1.3	

Table XXII (continued)

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (% or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p.p.m.) and plant parts analyzed	
								fruit	
						7		0.65	
						7		0.68	
						14		0.18	
						14		0.75	
						21		0.94	
						21		1.00	
		G.A.	4 lb. of 25 EC 120 g. a.i./100 l.	As above	2	0	GC	3.4	
						0		4.5	
						3		1.8	
						3		2.4	
						7		1.4	
						7		0.68	
						14		1.2	
						14		2.4	
						21		1.5	
						21		2.5	
								fruit	*dried fruit*
Apricots Blenheim	U.S.A. Calif. (1966)	G.A.	2 lb. of 25 EC 60 g. a.i./100 l. +surfactant (8 oz. X-77/100 gal.)	13. VI./9. VII. growth stage of the fruits: nearly mature spray mixture: 4—500 gal./acre	2	0	GC	2.7	
						0		3.1	
						2		1.8	
						2		1.3	
						7		0.5	
						7		0.9	

						14			0.37	
						14			0.53	
						21			0.29	
						21			0.28	
			4 lb. of 25 EC 120 g. a.i./100 l. +surfactant (8 oz. X-77/100 gal.)	As above	2	0	GC		7.1	
						0			5.8	
						2			3.6	
						2			6.7	
						7			1.5	
						7			1.8	
						14			1.6	
						14			1.8	
						21			0.87	
						21			1.2	
Prunes Imperial	U.S.A. Calif. (1966)	G.A.	2 lb. of 25 EC 60 g. a.i./100 l. +surfactant (8 oz. X-77/100 gal.)	9. VII./5. VIII. growth stage of the fruits: mature	2	0	GC		0.60	
						0			0.69	
						3			0.78	
						3			0.65	
						8			0.59	
						14			0.71	
						14			0.48	
						21			0.61	0.19
						21			0.92	—
									fruits	
Prunes Imperial	U.S.A. Calif. (1966)	G.A.	4 lb. of 25 EC 120 g. a.i./100 l. +surfactant (8 oz. X-77/100 gal.)	9. VII./5. VIII growth stage of the fruits: mature	2	0	GC		3.2	
						0			4.6	
						3			1.3	
						3			1.1	
						8			1.0	
						8			1.5	
						14			0.9	
						14			0.73	
						21			0.82	
						21			0.50	

Table XXII (continued)

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (%/o or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p.p.m.) and plant parts analyzed	
Plums President	U.S.A. Calif. (1966)	G.A.	2 lb. of 25 EC 60 g. a.i./100 l.	16. VI./22. VII. growth stage of the fruits: mature spray mixture: 300—500 gal./acre	2	0	GC		*fruits* 1.8
						0			1.4
						3			1.3
						3			1.3
						7			1.3
						7			1.6
						14			1.9
						14			2.1
						21			1.1
						21			1.1
		G.A.	4 lb. of 25 EC 120 g. a.i./100 l.	—	2	0	GC		3.2
						0			3.4
						3			2.8
						3			2.6
						7			3.0
						7			3.1
						14			2.6
						14			2.9
						21			1.9
						21			2.4
								skin	*meat*
Grapefruit	U.S.A. Florida (1965)	G.A.	1 lb. of 25 EC 30 g. a.i./100 l.	30. VIII. spray mixture: 15 gal./tree	1	0	GC	1.6	< 0.1
						0		2.6	< 0.1
						7		2.1	< 0.1
						7		5.5	< 0.1

						14		1.1	< 0.1
						14		3.5	< 0.1
						21		1.6	< 0.1
						21		0.75	< 0.1
		G.A.	2 lb. of 25 EC 60 g. a.i./100 l.	As above	1	0		5.8	< 0.1
						0		11.0	< 0.1
						7		2.6	< 0.1
						7		3.9	< 0.1
						14		5.8	< 0.1
						14		4.0	< 0.1
						21		3.6	< 0.1
						21		3.2	< 0.1
		G.A.	4 lb. of 25 EC 120 g. a.i./100 l.	As above	1	0		14.0	0.16
						0		11.0	< 0.1
						7		9.4	< 0.1
						7		14.0	< 0.1
						14		7.4	< 0.1
						14		5.5	< 0.1
						21		7.2	< 0.1
						21		4.8	< 0.1
								peel	*pulp*
Lemons	U.S.A. Calif. (1965)	G.A.	1.5 lb of 25 EC 45 g. a.i./100 l.	30. VIII. with hand gun spray mixture: 2,000 gal./acre	1	1	GC	5.9	< 0.1
						1		6.5	—
						8		5.9	< 0.1
						8		5.8	—
						14		6.8	< 0.1
						21		6.1	< 0.1
		G.A.	3 lb. of 25 EC 90 g. a.i./100 l.	As above	1	1	GC	14.0	< 0.1
						1		15.0	—
						8		7.4	< 0.1
						8		7.1	—
						14		10.7	< 0.1
						21		8.8	< 0.1

Table XXII (continued)

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (%₀ or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p.p.m.) and plant parts analyzed	
								peel	*pulp*
Oranges	U.S.A. Florida (1965)	G.A.	1 lb. of 25 EC 30 g. a.i./100 l.	20. XI. growth stage of the fruit: mature	1	0	GC	0.55	< 0.04
						14		0.59	< 0.04
						21		0.70	< 0.04
						40		0.39	< 0.04
		G.A.	2 lb. of 25 EC 60 g. a.i./100 l.	As above	1	0	GC	0.42	< 0.04
						14		0.50	< 0.04
						21		0.84	< 0.04
						40		1.1	< 0.04
		G.A.	4 lb. of 25 EC 120 g. a.i./100 l.	As above	1	0	GC	1.0	< 0.04
						14		0.76	< 0.04
						21		0.86	< 0.04
						40		0.79	< 0.04
									fruit
Grapes Thompson seedless	U.S.A. Calif. (1966)	G.A.	2 lb. of 25 EC 60 g. a.i./100 l.	5. VIII./2. IX. growth stage of the fruit: mature spray mixture: 200 gal./acre	2	0	GC		1.5
						0			2.3
						3			2.2
						3			1.4
						7			2.5
						7			1.4
						17			0.56
						17			0.93
						28			< 0.10
						28			0.95

		G.A.	4 lb. of 25 EC 120 g. a.i./100 l.	As above	2	0	GC		1.2	
						0			2.4	
						3			2.2	
						3			1.5	
						7			0.39	
						7			0.68	
						17			2.5	
						17			5.2	
						28			2.5	
						28			1.5	
									dried leaves	*fermented leaves*
Tea	Indonesia Java (1968)	G.B.	0.05% of 50 WP 25 g. a.i./100 l.	spray mixture: 400 l./ha.	1	1	GC		19.0	14.0
						1			19.0	
						4			6.7	4.8
						4			4.4	
						7			3.6	1.8
						7			7.1	
						9			< 0.2	0.3
						9			< 0.2	
		G.B.	0.1% of 50 WP 50 g. a.i./100 l.	As above	1	1	GC		33.0	20.0
						1			33.0	
						4			8.9	13.0
						4			7.4	
						7			14.0	4.7
						7			18.0	
						9			2.5	0.2
						9			2.0	
Tea *Albizzia odoratissima*	India Assam (1969)	G.A.	1.25 l. of 25 E/ha. 312 g. of a.i./ha.	spray mixture: 100 l./ha.	1	7	GC		*manufactured dried leaves*	
									unmixed	*mixed* [d]
									2.6	1.0

Table XXII (continued)

Crop and variety	Country and year	Ref. [a]	Dosage and formulation [b] (%/o or lb./100 U.S. gal. or/acre) and (g. a.i./100 l. or kg. a.i./ha.)	Application dates	No. of appl.	Interval between last appl. and sampling (days)	Anal. method [c]	Residues (p.p.m.) and plant parts analyzed	
								brewed tea	
								wet leaves	*brew*
								2.05	< 0.01
Cucumbers Butchers disease resistant	U.K. (1963)	Ch.E.	0.1% of 25 EC 25 g. a.i./100 l.	22. VIII.	1	1	Col.	0.86	
				2. X.	1	2	Col.	0.73	
Cucumbers	Switzer-land (1963)	G.B.	0.1% of 25 EC 25 g. a.i./100 l.	greenhouse application	1	1	GC	4.0	
						5		2.0	
						8		0.6	
						12		0.4	
						15		0.3	
						19		0.2	
Muskmelons	U.S.A. N. York (1965)	G.A.	1 lb. of 50 WP 0.56 kg. a.i./100 l.	20. IX. foliage spray spray mixture: 150 gal./acre	1	0	GC	0.18	
						0		0.13	
						7		< 0.05	
						7		< 0.05	
						14		< 0.05	
						14		< 0.05	
		G.A.	2 lb. of 50 WP 1.12 kg. a.i./100 l.	As above	1	0	GC	0.16	
						0		0.11	
						7		0.11	
						7		0.06	
						14		0.09	
						14		< 0.05	

		G.A.	4 lb. of 50 WP 2.24 kg. a.i./100 l.	As above	1	0	GC		0.36
						0			0.07
						7			0.09
						7			0.08
						14			0.08
						14			0.09
									fruit
Tomatoes Rutgers	U.S.A. N. Y. (1965)	G.A.	1 lb. of 50 WP 0.56 kg a.i./ha.	foliage spraying 150 gal./acre 10. IX.	1	3	GC		0.11
						3			0.12
						7			< 0.05
						7			0.12
						14			0.05
						14			< 0.05
		G.A.	2 lb. of 50 WP 1.12 kg. a.i./ha.	As above	1	3	GC		0.08
						3			0.06
						7			0.07
						7			0.06
						14			< 0.05
						14			0.13
		G.A.	4 lb. of 50 WP 2.24 kg. a.i./ha.	As above	1	3	GC		0.10
						3			0.14
						7			0.17
						7			0.10
						14			0.06
						14			0.11

and apple meat) [4]. None of the treated apples contained detectable amounts of 4,4′-dichlorobenzilic acid.

In culture media chloropropylate is metabolized by microorganisms. Over 300 microorganisms were tested. Among these, especially yeast (*Rhodotorula gracilis* Rennerfelt) is able to degrade chloropropylate in to two main and several intermediate metabolites in a mineral medium supplemented by sucrose. The conversion is presumably due to hydrolysis to 4,4′-dichlorobenzilic acid and then to decarboxylation, as well as dehydrogenation to 4,4′-dichlorobenzophenone. The carboxylesterase is more active with ethyl esters than with isopropyl, which is why chloropropylate is metabolized to a lesser degree than chlorobenzilate. On account of storage of the product in the fat of yeast cells there is no complete degradation as is also the case with chlorobenzilate (MIYAZAKI *et al.* 1970).

c) Tolerances and waiting periods

Tolerances for chloropropylate have been established in the U.S only. It is five p.p.m. for apples and pears. Temporary tolerances (to be in effect until 1972) were proposed by FAO/WHO in 1968 for the following raw agricultural products:

3 p.p.m. for apples, pears, citrus fruits (whole fruit basis)
1 p.p.m. for tomatoes, cantaloupes

Waiting periods (days between last application and harvest) for chloropropylate applications are 14 days for apples and pears in the U.S., and seven days in France.

XV. Analysis of chloropropylate

a) Analysis of active ingredient and formulations

1. Review of methods. — Chloropropylate can be analyzed by determination of total chlorine as described by STEPANOW (1906), CARTER and HUBANKS (1946), CARTER (1947), and CARTER *et al.* 1950). The saponification of the ester group and the acetylation of the hydroxy group for chloropropylate assay is described by SUTER (1963). DAVIS *et al.* (1968) described an infrared procedure for the determination of chloropropylate. SUTER *et al.* (1963), DAVIS *et al.* (1968), and BAILEY (1965) developed gas chromato-

[4] Apple trees were sprayed with two lbs. a.i./100 gal.; samples were taken three, 14 and 21 days after application. The sensitivity of the analytical method was 0.1 p.p.m. The recovery values indicate the ability to detect the 4,4′-dichlorobenzilic acid with no interference from chloropropylate. Extraction studies on field-weathered 14-day samples showed that unchanged chloropropylate was present at 89 percent on the surface of the apple and not in the apple meat. The loss of 11 percent can be attributed to volatization from the apple surface into the atmosphere. Chloropropylate injected into the apple meat (two mg. a.i., dissolved in acetone) was recoverable at 97 percent and was unchanged after 18 days.

graphic methods. The internal standard GLC-method is highly recommended for the analysis of active ingredient and formulations.

2. Analysis of technical chloropropylate. —

α) *Total chlorine content.* —

APPARATUS:

200-ml. ground joint Erlenmeyer flask
Reflux condenser, with ground glass joint

REAGENTS:

Metallic sodium
Isopropyl alcohol, redistilled, free of organic chlorine
Isopropyl alcohol, 70 percent in water
Sulfuric acid, pure, 50 percent
Silver nitrate solution, 0.1 N

PROCEDURE:

Weigh 0.29 to 0.32 g. of chloropropylate into a 200-ml. ground joint Erlenmeyer flask, add 50 ml. of isopropanol and about five g. of sodium metal cut into small cubes. Connect with a dry reflux condenser and keep slightly boiling for at least one hour. Destroy the excess of sodium metal by introducing, drop by drop, a few ml. of the 70 percent isopropanol through the condenser. Boil again for a few minutes and add 50 ml. of distilled water. After cooling, transfer the solution into a 250-ml. beaker and rinse the Erlenmeyer with a few ml. of water. Acidify slightly with 50 percent sulfuric acid and titrate potentiometrically with 0.1*N* silver nitrate solution, using electrodes Ag and Hg/Hg_2SO_4 with a K_2SO_4 bridge.

ml 0.1 N silver nitrate solution consumed = a
weight of sample in g = w

$$\text{Calculation: } \frac{a \times 339.2 \times 100}{w \times 20{,}000} = \text{percent chloropropylate}$$

Ionic chlorine has to be determinated separately as follows and subtracted in the calculation:

Dissolve a three to six g. sample of chloropropylate in 50 ml. of isopropanol, dilute with 50 ml. of water, acidify with 50 percent sulfuric acid, and titrate as above.

β) *Saponification.* —

APPARATUS:

200-ml. ground joint Erlenmeyer flask
Reflux condenser with ground glass joint

REAGENTS:

Neutral ethanol
Potassium hydroxide, 0.5 N solution in ethanol

Hydrochloric acid, 0.5 N solution
Phenolphthalein, 0.1 percent solution in 60 percent ethanol

PROCEDURE:

Weigh four to five g. of chloropropylate into a 200-ml. ground joint Erlenmeyer flask and add 50 ml. of ethanol. Neutralize, after addition of some drops of phenolphthalein solution, with 0.5*N* ethanolic potassium hydroxide solution to red. Add 50.0 ml. of 0.5*N* ethanolic potassium hydroxide solution, and reflux for one hour. Rinse the condenser with a few ml. of neutral ethanol and, after addition of some further drops of the indicator, titrate the excess of alkali with 0.5*N* hydrochloric acid while the solution is still hot.

Weight of sample in g. = w
ml 0.5 N hydrochloric acid consumed = a

In the same way run a blank test with 50.0 ml. of 0.5*N* ethanolic potassium hydroxide solution and titrate with 0.5*N* hydrochloric acid as before.

ml 0.5 N hydrochloric acid consumed = b

$$\text{Calculation: } \frac{(b-a)\times 339.2\times 100}{w\times 2{,}000} = \text{percent chloropropylate}$$

γ) Gas chromatography (internal standard method). —

RECOMMENDED INSTRUMENT PARAMETERS:

Instrument: Aerograph, model 1200-1

Integrator: Infotronics, model CRS-104

Recorder: Sargent, 2.5 mV, chart speed 1.2 cm./minute

Column: 1 m.×3.5 mm. with 5 percent OV 25 Silicone on Chromosorb W AW DMCS HP 80/100 mesh.

Detector: flame ionization detector

Temperature: column oven — 210 °C. isothermal
detector — 210 °C.
injector — 240 °C.

Carrier gas: helium — 60 ml./minute
hydrogen — 60 ml./minute
air — 600 ml./minute

Sample solution: 1 g. of chloropropylate and 1 g. of internal standard (succinic acid dibenzylester) both analytically weighed are dissolved in 10 ml. of methylene chloride.

Volume injected: 0.5 µl.

Relative retention times: chloropropylate — 1.00
standard — 1.58

Total time for chromatogram: 20 minutes

Recorder set: sensitivity accordingly adjusted to give 60 to 80 percent response

CALCULATIONS:

$$\% \text{ chloropropylate} = \frac{G_{St} \cdot F_A \cdot f \cdot 100}{G_A \cdot F_{St}}$$

G_{St} = weight of standard in g.
G_A = weight of chloropropylate in g.
F_{St} = peak area of standard in counts
F_A = peak area of chloropropylate
f = specific factor, numerical value about 1.2; has to be determined at each analysis with a reference sample.

Precision: ± 1 percent at 95 percent confidence limits for duplicates.

3. Analysis of formulations. —

α) Principle. — Chloropropylate in emulsifiable solutions and wettable powders is analyzed by the determination of the total chlorine content or by saponification as described for the analysis of technical materials. In the case of wettable powders the active ingredient is extracted by ethyl ether, whereas emulsions can be directly analyzed.

β) Procedure. — Weigh accurately an amount of product containing between 0.29 and 0.31 g. of chloropropylate and extract in a Soxhlet apparatus with ethyl ether during eight hours. Evaporate to dryness and dissolve the residue in 50 ml. of isopropanol. Continue as outlined for the analysis of technical materials.

γ) Discussion of method. — The method described is suitable for all formulations. For emulsifiable solutions extraction is not necessary. For wettable powders with a relatively high content in active ingredient combustion with sodium peroxide in the Parr or Wurzschmitt bomb is somewhat more rapid (WURZSCHMITT 1950 a and b, 1951).

b) Residue analysis

1. Extraction. — Only few procedures are reported in the literature for the extraction of chloropropylate from crop samples. Because of the chemical similarity with chlorobenzilate the method described would be equally satisfactory for chloropropylate residues, providing that it is not necessary to distinguish between them.

Chloropropylate is extracted from chopped materials with petroleum ether (boiling range 30° to 60° C.) (WHO and FAO method 1969). Apples, pears, stone-fruits, almonds, cucumbers, and beans were analyzed for chloropropylate. Methanol was also used (DELLEY *et al.* 1964) for the removal of chloropropylate from apples, pears, and cucumbers. A mixture of isopropyl alcohol and benzene was recommended as extraction solvent in the maceration of sprayed apples, tomatoes, and cucumbers (BENFIELD and RICHARDSON 1965). Interferences from DDT were removed by hydrolysis and solvent extraction.

2. Cleanup. — Depending on the final determination method there are several alternatives. A very effective cleanup for separating chloropropylate

from interfering material was developed by DELLEY *et al.*(1964) and successfully used in a variety of food and animal feed products. Column chromatography has extensively been used as a general cleanup technique.

Alumina is a well-known adsorbent for the removal of interfering materials like waxes, oils, and pigments, widely used for column chromatography. This adsorbent was the only one used for cleanup before gas chromatographic detection (WHO and FAO 1969). Interfering materials were removed from the basic alumina column by eluting with hexane or with 10 percent benzene in hexane. Chloropropylate was eluted with benzene/hexane (1:1). For oily samples such as nuts, seeds, and citrus peel an additional petroleum ether/acetonitrile partitioning step is necessary. If this cleanup procedure is applied, no interference from DDT and DDD (TDE) is encountered.

Chromatographic columns filled with Florisil gave satisfactory separation of chloropropylate from interfering plant materials when eluted with 50 ml. of diethyl ether prior to the gas chromatographic detection with the microcoulometric titration cell sensitive to chlorine.

Further cleanup is necessary to reduce the background for the determination of chloropropylate by the flame ionization detector. A silica gel cleanup column was used, with *p*-phenylazophenol as an indicator and dichloromethane with two percent methanol as the eluent. A small decolorizing carbon column was attached to remove the *p*-phenylazophenol indicator from the cleaned chloropropylate.

3. Methods of determination. —

α) Spectrometric method. — Chloropropylate residues in apples, tomatoes, and cucumbers have been determined by a photometric method (BENFIELD and RICHARDSON 1965). The method of HARRIS (1965) for chlorobenzilate, consisting of nitration and colorimetric determination at 540 nm, was adapted, usig five percent potassium hydroxide in methanol for the final colour development.

β) Gas chromatographic methods. — Gas chromatographic techniques for the detection of chloropropylate are quite common. Various detector systems were used to determine chloropropylate residues. The non-specific hydrogen flame ionization detector needs a rigorous cleanup of crop extracts prior to final determination (DELLEY *et al.* 1964). The column was glass, filled with 2.5 percent Reoplex 400 on Anakrom ABS and the column temperature was 170° C. About 0.1 μg. of parent compound could be detected.

A chloride specific microcoulometric detector, originated by COULSON *et al.* (1960), has found wide application in the analysis of residues of halogenated pesticides. Chloropropylate was determined (WHO and FAO 1969) with a microcoulometric detector and nitrogen as the carrier gas. The glass column (4 mm. diam. × 1 m.) was packed with five percent silicone gum GE XE-60 supported on Anakrom ABS (50/60 mesh).

The COULSON (1965 and 1966) electrolytic conductivity detector system, normally used for nitrogen detection, was also used successfully to determine halogenated compounds such as chloropropylate. The quartz reduction tube was packed with a platinium gauze rolled lengthwise. No absorber was placed in the exit end of the reduction tube. The column was glass, filled with five percent silicone rubber SE 30 on Chromosorb WS.

4. Recommended method. —

α) Principle. — The acaricide is extracted from the crop material by blending with acetone-hexane mixed solvent. The extract is chromatographed to remove the interfering materials. Quantitative measurements are made using a gas chromatograph with a microcoulometric titration cell sensitive to chloride.

REAGENTS:

Acetone: Reagent grade

Benzene: Reagent grade

n-Hexane: BR 65° to 60 °C

Aluminum oxide: Woelm, basic, activity grade I (Bodman Chemicals, Inc., 106 North Essex Ave., Narberth, Penn. 19072)

Aluminum oxide: Activity grade V. Prepared by mixing 85 g. of Woelm, basic alumina, activity grade I with 15 g. of water. The water and alumina are mixed thoroughly and allowed to stand overnight in a tightly closed bottle before use.

Acetonitrile: BR 80.5° to 82.5 °C.

APPARATUS:

The column is 18 mm. ID×200 mm. long and is equipped with a perforated support plate and a 100-ml. reservoir by joining a 100-ml. round bottom flask to the top.

Hobart Food Cutter — Catalog no. 84141 (The Hobart Manufacturing Co., Troy, Ohio)

Blender — Osterizer or equivalent

Flash evaporator — Büchi or equivalent

Vials — 2-dram capacity

Microcoulometric gas chromatograph — Model MT 220 (Micro Tek Instruments Corp., Austin, Texas). This instrument is equipped with a Dohrmann (San Carlos, Calif.) T-300-s microcoulometric titration cell sensitive to chloride, a C-200a microcoulometer and an S-200 combustion furnace.

Glass injection port — Micro Tek Instruments Corp. (Austin, Texas)

Syringes — 0.010-ml. sample injection syringe

Gas chromatography column — Glass column (2 feet×1/4 inch O.D.) packed with three percent by weight of Carbowax 20 M on 60/80 mesh Gas Chrom Q.

β) Extraction procedure. — A representative sample of 300 to 400 g. of the crop is chopped in a Hobart Food Cutter. A 100-g. sample is then transferred to a blender jar equipped with a plastic cap. Several untreated check samples are fortified by adding known amounts of the acaricide sought to the samples at this point. The amount added should be near the expected values for the treated samples. A polyethylene liner is placed under the cap to avoid dissolving extraneous material from the cap lid and to avoid loss of solvent. Exactly 400 ml. of a mixed solvent (hexane and acetone 3:1 by volume) are added to the sample. The sample and solvent are blended for 10 minutes, allowed to stand for two minutes and then blended again for one minute. Up to 100 g. of anhydrous sodium sulphate may be added to the blender to facilitate the extraction.

The blended sample is poured through a long-stem funnel containing a glass wool plug into a 500-ml. separatory funnel containing 250 ml. water and 25 ml. of a saturated sodium sulfate solution. The funnel is shaken vigorously for one minute and then allow to stand until the aqueous and organic layers separate. The aqueous (lower) phase is discarded and the remaining hexane phase is filtered through a one inch pad of anhydrous sodium sulfate and collected in a 16-oz. bottle.

γ) Alumina column cleanup. — A 30-ml. aliquot (equivalent to 10 g. of crop) of the hexane extract is transferred to a 250-ml. Erlenmeyer flask (equipped with a ground glass joint) and brought to dryness using a flash evaporator. The sample is now ready for column cleanup.

A dry packed column is prepared by adding 25 g. of alumina basic, activity V to a column. The column is then tapped gently to eliminate channeling and to achieve uniform packing of the alumina. A glass wool plug is placed on top of the alumina. The sample residue is dissolved in two ml. of benzene. This solution is transferred to the column and allowed to penetrate into the alumina. The flask is washed with five ml. of *n*-hexane which is transferred to the column and allowed to penetrate as before. This is repeated with five ml. of *n*-hexane. A 250-ml. Erlenmeyer flask is used as a receiver. When the last five ml. of wash *n*-hexane has just run into the column surface, 90 ml. of *n*-hexane is added and allowed to pass through the column. When the *n*-hexane has just run into the surface of the column, a clean 250-ml. flask is placed as receiver and 100 ml. of 1:1 hexane-benzene is added to the column and the elution is continued. The whole 100 ml. of eluant solution is then collected. The eluate is evaporated to dryness using a flash evaporator. The residue is transferred with hexane to a two-dram vial. The hexane is evaporated to dryness in a hot water bath with the aid of a gentle stream of dry air. A volume of 0.2 to 0.5 ml. of benzene is added to the residue, the vial is shaken to ensure complete solution of the residue, and is stoppered. The sample is now ready for gas chromatography.

Ten percent benzene in *n*-hexane has been used as the first eluant for some samples containing large amounts of interfering material. The acaricide is not eluted by this solvent mixture.

δ) Gas chromatography. —

Injector temperature — 225 °C.
Column temperature — 200 °C.
Transfer tube temperature — 250 °C.
Furnace temperature — 875 °C.
Carrier — Nitrogen, 110 ml./min.
O_2 flow — 50 ml./minute
N_2 purge — 20 ml./minute
Attenuation — 200 ohms
Titration cell — T-300-s (Chloride specific)
Minimum detection limit — 10 ng.
Injection volume — 2 to 8 µl.
Chart speed — 0.5 inch/minute
Retention time — chloropropylate 2.5 minutes

The gas chromatograph response is standardized by injecting known amounts of analytical standard. A stock solution is prepared by dissolving 100 mg. of standard in 100 ml. of benzene from which an aliquot is diluted until a final concentration of 10 ng./µl. is reached. Volumes of two to eight µl. (equivalent to 20 to 80 ng.) are injected. Peak areas are determined by triangulation and standard curves are constructed by plotting ng. of acaricide standard injected against peak areas.

Recoveries of acaricide standards added to the check samples before extraction and carried through the whole analytical procedure are determined. The amount of acaricide recovered (ng.) is read from the standard curve from the peak areas obtained from the fortified samples. The p.p.m. values are then calculated by dividing the ng. of acaricide by the weight (mg.) of the crop equivalent injected. The percent recovery is then calculated based on the theoretical value added.

For unkown samples, the analyses and calculations are done as described above. In addition, corrections are made for the percent recovery obtained from the recovery studies.

5. Special procedure for oily samples. — Samples such as nuts, cottonseed, and citrus peel require separation of the oil before column chromatography. The hexane extract is evaporated on a steam bath to a volume of 50 ml. It is then transferred to a 250-ml. separatory funnel and the beaker is washed twice with 25-ml. portions of hexane. The acaricide is extracted from the hexane solution using two 25-ml. portions of acetonitrile. The combined acetonitrile extract is then washed with 50 ml. of hexane to remove traces of oil carried along. The acetonitrile containing the acaricide is transferred quantitatively into a 100-ml. round-bottom flask equipped with a 24/40 joint.

The acetonitrile is completely evaporated under reduced pressure (water aspirator) using a flash evaporator with the water bath at 50° C. The sample is now ready for chromatographic cleanup, as described previously.

6. Samples of unknown history. — Practically all of the samples analyzed in our laboratory are samples whose history of treatment with

pesticides is known. Some observations can, however, be made in regard to specificity of the methods described.

The chloride sensitive detector eliminates interference from non-halogenated compounds.

Interference from DDT and DDD (TDE) is eliminated by the cleanup procedure. These materials are eluted in the hexane fraction when using the alumina column and so are removed before gas chromatography. The DDT and DDD (TDE) content could be determined independently if so desired.

Specificity is also given by the elution times. Chlorobenzilate and chloropropylate peaks are resolved by the gas chromatographic column described above. Conversely if separation is not required, these acaricides have been gas chromatographed successfully using the following liquid phases: GE-Nitrile XE-60, SE-30, and QF-1.

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Summary

The two carbinole compounds chlorobenzilate and chloropropylate were synthesized in the laboratories of *J. R. Geigy S. A.* in the early 1950's and displayed a specific acaricidal activity already in the first tests. Consequently, chlorobenzilate was introduced on the market as a specific chemical for the control of spider mites in 1952. It was not until 10 to 12 years later that chloropropylate was developed further, since it proved to be an excellent acaricide for the control in fruit growing of mite strains that had become increasingly resistant to organic phosphates.

In this review all pertinent published and unpublished data on the toxicology, metabolism, mode of action, application fields, and residues of chlorobenzilate and chloropropylate are assembled and presented. In addition, analytical methods for the determination of residues on food commodities and of the active ingredients in formulations are described.

The acute oral LD_{50} of chlorobenzilate in rats is in the order of magnitude of approximately 700 to 3,100 mg./kg. depending on the solvent used; the acute oral LD_{50} of chloropropylate in rats was found to be greater than 5,000 mg./kg. The dermal toxicity of both products is very low. In two-year chronic feeding studies in rats 40 p.p.m. (=2.5 mg./kg./day) of each product proved to be a no-effect level. In similar two-year feeding studies in dogs 100 and 500 p.p.m. of chlorobenzilate (=2.7 and 12.6 mg./kg./day, respectively) as well as of chloropropylate (= 3.5 and 17.6 mg./kg./day, respectively) were found to be no-effect levels. In comparative tests both products were of a much lower toxicity to birds and fish than DDT. In

metabolism studies on dogs it could be shown that the greater part of the administered chlorobenzilate and chloropropylate was rapidly excreted in the urine. Dichlorobenzilic acid was the only metabolite to be detected by thin-layer chromatography.

The acaricides chlorobenzilate and chloropropylate are effective against susceptible and resistant mite genera of *Tetranychidae*, *Eriophyidae*, and *Phytoptipalpidae* in orchards, vineyards, citrus- and tea plantations, and in field crops, as well as on ornamental plants. In addition, chlorobenzilate has been successfully used against the tracheal mite of the bee. Treatments at dosages of 30 to 45 g. of a.i./100 liters are most successful when the first mite damages on the crops occur. Since both products are contact acaricides, spraying must be carried out very thoroughly so that the entire surface of the plant is wetted. Treatments with these selective acaricides do not affect the useful insects. Predaceous mites are only little susceptible. Both products are harmless to bees at any time.

The products are well tolerated with the exception of some stone fruit varieties (mainly peaches and plums). Chloropropylate is by far better tolerated by stone fruit than chlorobenzilate.

Fruits, citrus, grapes, tea, and vegetables, treated according to good agricultural practice, were analyzed for residues. The residue levels found some days after the last treatment are below the temporary tolerances proposed by FAO/WHO. Fourteen days after the last application approximately 1.5 p.p.m. are found on fruits, 2.6 p.p.m. on citrus (peel), 1.0 p.p.m. on grapes, 0.25 p.p.m. on vegetables, and seven days after the last application >3.0 p.p.m. on tea.

The products are metabolized by some microorganisms but not in or on plants. They are translocated into the plant soon after application.

The tolerances established in various countries for chlorobenzilate and chloropropylate on raw agricultural commodities range between 0.2 and eight p.p.m., and are mostly five p.p.m. The prescribed or recommended intervals between the last application and harvesting are 14 days for pip-fruits and seven days for stone fruits.

Analytical methods for the determination of residues and active ingredient of chlorobenzilate and chloropropylate, respectively, in formulations are reviewed and presented. The technical materials may be analyzed by determination of the total chlorine content or by saponification of the ester group or by a gas chromatographic method. The methods used are suitable for both types of formulations: emulsifiable concentrates and wettable powders.

Extraction and cleanup procedures for residue analysis followed by gas chromatographic and spectrophotometric determination are reviewed. An improved method for residue analysis is recommended. In this method the active ingredient is extracted from crop material with acetone/hexane. The extract is chromatographed on an alumina column to remove interfering materials. Quantitative measurements are made, using a gas chromatograph with a microcoulometric titration cell sensitive to chloride.

Résumé *
Le chlorobenzilate et le chloropropylate, acaricides à base de carbinole

Les deux carbinoles chlorobenzilate et chloropropylate furent synthétisés au début des années 50 dans les laboratoires de J. R. Geigy S. A. et montrèrent dès les premiers tests qu'ils étaient des acaricides spécifiques. Le chlorobenzilate a par conséquent été introduit sur le marché en 1952 comme produit spécial de lutte contre les araignées rouges. Le chloropropylate n'a été mis au point que 10–12 ans plus tard, car il a prouvé être extrêmement efficace en arboriculture contre les araignées rouges devenues de plus en plus résistantes aux esters phosphoriques.

Le présent travail mentionne toutes les données, publiées ou non, se rapportant à la toxicologie, au métabolisme, au mode d'action, à l'application et aux résidus de chlorobenzilate et de chloropropylate. Il traite en outre des méthodes d'analyse des résidus de ces deux substances dans des échantillons biologiques et de la détermination des matières actives dans les produits formulés. La DL_{50} du chlorobenzilate chez le rat, selon la forme d'administration, est comprise entre environ 700 et 3100 mg/kg; la DL_{50} du chloropropylate est supérieure à 5000 mg/kg. Les deux substances sont caractérisées par une faible toxicité cutanée. Des essais de 2 ans d'alimentation chronique ont montré que la dose de 40 ppm (millionièmes), soit 2,5 mg/kg/jour, est sans effet toxicologique sur les rats. Chez les chiens, lors d'essais d'administration chronique de 2 ans, il s'est avéré que 100 et 500 ppm de chlorobenzilate (= 2,7 ou 12,6 mg/kg/jour) et de chloropropylate (= 3,5 ou 17,6 mg/kg/jour) représentent un seuil d'effet nul. Pour les oiseaux et les poissons, ces deux acaricides ont été nettement moins toxiques que la matière active DDT, également incluse dans les essais à titre de comparaison. Des études de métabolisme sur le chien ont montré que la majeure partie du chlorobenzilate ou du chloropropylate administré est excrétée dans l'urine. Le seul métabolite identifié est l'acide dichlorobenzilique.

Le chlorobenzilate et le chloropropylate sont des acaricides efficaces contre les acariens des familles Tetranychidae, Eriophyidae et Phytoptipalpidae s'attaquant aux arbres fruitiers, à la vigne, aux agrumes, au théier et aux grandes cultures, ainsi qu'aux plantes ornementales. La matière active chlorobenzilate est en outre utilisée avec succès dans la lutte contre l'acariose de l'abeille.

Le dosage le plus efficace est de 30–45 g de m.a./100 l dès la première apparition des dégâts. En cas de forte attaque, il est indiqué de faire une deuxième application 7–10 jours après la première. Tous deux sont des acaricides de contact, de sorte que toute la plante doit être bien mouillée par la bouillie au moment du traitement. Les traitements faits avec ces deux acaricides spécifiques n'ont pas d'action néfaste sur la faune utile, formée principalement par des insectes. Les acariens prédateurs ne sont que peu

* Traduit par R. TRUAN.

sensibles à ces deux produits qui sont en outre sans danger pour les abeilles, quelle que soit l'époque du traitement.

Le chlorobenzilate et le chloropropylate sont bien tolérés par les plantes, à l'exception de quelques variétés de fruits à noyau (surtout des pêchers et des pruniers). Le chloropropylate est mieux toléré que le chlorobenzilate par les arbres fruitiers à noyau.

Des analyses de résidus ont été faites après des traitements usuels de la pratique sur arbres fruitiers, agrumes, vigne, théier et cultures maraîchères. Quelques jours après l'application, les résidus identifiés étaient inférieurs aux tolérances provisoires proposées par la FAO/WHO. Quatorze jours après le dernier traitement, on a décelé environ 1,5 ppm sur les fruits, 2,6 ppm sur les agrumes (écorce), 1 ppm sur les raisins, 0,25 ppm sur les légumes et, sur les feuilles de thé >3 ppm au bout de 7 jours.

Les deux produits sont métabolisés par quelques micro-organismes, mais non sur ou dans les végétaux. Il y a translocation dans la plante peu après l'application.

Les tolérances fixées pour le chlorobenzilate et le chloropropylate dans divers pays et pour les produits des récoltes et les vivres d'origine végétale — comprises entre 0,2 et 8 ppm — sont généralement de 5 ppm. Les délais d'attente imposés ou recommandés entre la dernière application et la récolte sont de 14 jours pour les fruits à pépins et de 7 jours pour les fruits à noyau.

Cette étude mentionne et décrit les méthodes d'analyse des résidus de chlorobenzilate et de chloropropylate dans du matériel biologique et la détermination de la matière active dans les produits formulés.

Le chlorobenzilate et le chloropropylate techniques peuvent être analysés par la détermination de la teneur totale en chlore ou par saponification du groupe ester ou par chromatographie en phase gazeuse. Les méthodes utilisées conviennent aussi bien à l'analyse des solutions émulsionnables qu'à celle des poudres mouillables.

Il est aussi fait mention des procédés d'extraction et de purification pour l'analyse des résidus avec détermination subséquente par chromatographie en phase gazeuse et par spectrophotométrie. On recommande une méthode d'analyse qui a fait ses preuves dans la détermination des résidus. Selon cette technique, la matière active est extraite du matériel examiné au moyen d'acétone/hexane. L'extrait est purifié par chromatographie dans une colonne à oxyde d'aluminium. La détermination quantitative est effectuée par chromatographie en phase gazeuse avec une cellule microcoulométrique sensible au chlorure.

Zusammenfassung *

Die Karbinolakarizide: Chlorbenzilat und Chlorpropylat

Die anfangs der 50er Jahre in den Laboratorien der J. R. Geigy A. G. synthetisierten Karbinolverbindungen Chlorbenzilat und Chlorpropylat

* Verfaßt von den Autoren.

zeichneten sich bereits in den ersten Testversuchen durch eine spezifische akarizide Wirkung aus, so daß Chlorbenzilat als Spezialprodukt zur Spinnmilbenbekämpfung 1952 auf den Markt eingeführt wurde. Chlorpropylat wurde dagegen erst 10–12 Jahre später weiterentwickelt, da es sich als ausgezeichnetes Mittel zur Bekämpfung von Obstbau-Spinnmilben erwies, die gegenüber Phosphorsäureestern in immer stärkerem Maße resistent geworden waren.

In der vorliegenden Übersicht werden alle einschlägigen publizierten und unpublizierten Daten über Toxikologie, Metabolismus, Wirkungsmechanismus, Anwendung und Rückstände von Chlorbenzilat und Chlorpropylat angeführt. Des weitern werden analytische Methoden zur Rückstandsbestimmung von Chlorbenzilat und Chlorpropylat in biologischem Material und zur Wirksubstanzbestimmung in Formulierungen beschrieben. Die akute orale DL_{50} von Chlorbenzilat an der Ratte bewegt sich je nach Applikationsform in einem Bereich von ca. 700–3100 mg/kg; diejenige von Chlorpropylat liegt über 5000 mg/kg. Beide Produkte zeichnen sich durch eine geringe dermale Toxizität aus. In 2-jährigen chronischen Fütterungsversuchen an Ratten erwiesen sich 40 ppm (= 2,5 mg/kg/Tag) bei jedem Produkt als toxikologisch unwirksame Dosis. In 2-jährigen chronischen Versuchen an Hunden stellten 100 und 500 ppm sowohl beim Chlorbenzilat (= 2,7 bzw. 12,6 mg/kg/Tag) als auch beim Chlorpropylat (= 3,5 bzw. 17,6 mg/kg/Tag) „no-effect levels“ dar. Für Vögel und Fische erwiesen sich beide Akarizide im Vergleich zu dem ebenfalls mitgeprüften DDT-Wirkstoff als bedeutend weniger toxisch. In Metabolismusstudien am Hund konnte gezeigt werden, daß der größte Teil des verabfolgten Chlorbenzilats bzw. Chlorpropylats mit dem Urin ausgeschieden wird. Als einziger Metabolit wurde Dichlorbenzilsäure nachgewiesen.

Die Akarizide Chlorbenzilat und Chlorpropylat sind wirksam gegen normalsensible und resistente Milben der Familien der Tetranychidae, Eiophyidae und Phytoptipalpidae an Obst-, Wein-, Zitrus-, Tee- und Feldkulturen sowie an Zierpflanzen. Der Wirkstoff Chlorbenzilat wird außerdem mit gutem Erfolg zur Bekämpfung der Milbenseuche bei Bienen angewandt. Behandlungen mit 30—45 g AS/100 l beim ersten Erscheinen von Milbenschäden sind am erfolgversprechendsten. Bei starkem Befall sollte nach 7–10 Tagen eine zweite Applikation folgen. Da beide Produkte Kontaktakarizide sind, empfiehlt sich eine gute Benetzung der ganzen Pflanze. Bei Behandlungen mit diesen beiden spezifischen Akariziden wird die vorwiegend aus Insekten bestehende Nützlingsfauna nicht beeinträchtigt. Raubmilben sind nur wenig empfindlich. Beide Produkte sind für Bienen ungefährlich, gleichgültig zu welchem Zeitpunkt die Applikation erfolgt.

Die Produkte werden mit Ausnahme einiger Steinfruchtvarietäten (hauptsächlich Pfirsiche und Pflaumen) gut toleriert. Für Steinobst ist Chlorpropylat das besser verträgliche Produkt.

Obst, Zitrus, Reben, Tee und Gemüse wurden nach praxisüblichen Behandlungen auf Rückstände untersucht. Einige Tage nach der Applikation

lagen die Rückstände unter den von der FAO/WHO vorgeschlagenen temporären Toleranzen. Vierzehn Tage nach der letzten Behandlung ließen sich auf Obst etwa 1,5 ppm, auf Zitrus (Schale) 2,6 ppm, auf Trauben 1 ppm, auf Gemüse 0,25 ppm und auf Tee nach 7 Tagen >3 ppm feststellen.

Die Produkte werden von einigen Mikroorganismen, nicht aber auf oder in pflanzlichem Material metabolisiert. Bald nach der Anwendung werden sie in die Pflanze transloziert.

Die in verschiedenen Ländern festgelegten Toleranzen für Chlorbenzilat und Chlorpropylat auf pflanzlichen Ernteprodukten und Lebensmitteln pflanzlicher Herkunft liegen zwischen 0,2 und 8 ppm und betragen meistens 5 ppm. Die vorgeschriebenen oder empfohlenen Wartezeiten von der letzten Anwendung bis zur Ernte betragen für Kernobst 14 und für Steinobst 7 Tage.

Analytische Methoden zur Rückstandsbestimmung von Chlorbenzilat und Chlorpropylat in biolog. Material und zur Wirksubstanzbestimmung in Formulierungen werden zitiert und beschrieben.

Technisches Chlorbenzilat bzw. Chlorpropylat können durch Bestimmung des Gesamtchlorgehalts oder durch Verseifung der Estergruppe oder gaschromatographisch analysiert werden. Die verwendeten Methoden sind sowohl für emulgierbare Lösungen als auch für benetzbare Pulver geeignet.

Extraktion und Reinigungsverfahren für die Rückstandsanalyse mit anschließender gaschromatographischer oder spektrophotometrischer Bestimmung werden angeführt. Ein bewährtes Analysenverfahren für die Rückstandsbestimmung wird empfohlen. In diesem Verfahren wird die Aktivsubstanz aus dem Untersuchungsmaterial mit Azeton/Hexan extrahiert. Der Extrakt wird säulenchromatographisch an Aluminiumoxid gereinigt. Die quantitative Bestimmung erfolgt gaschromatographisch mit dem für Chlorid spezifischen Microcoulometer.

References

Ananthakrishnan, N. R.: Experiments on the control of tea mites in Southern India (1961–1962 field trials). Ann. Admin. Rept. United Planters Assoc. S. India, pp. XL–XLVIII (1961/62).

Anthon, E. W.: Peach silver mite control. J. Econ. Entomol. **47**, 866 (1954).

— Control of the plum nursery mite on sweet cherries. J. Econ. Entomol. **50**, 564 (1957).

Atkins, E. L., Jr., and L. D. Anderson: Toxicity of pesticide dusts to honeybees. J. Econ. Entomol. **47**, 969 (1954).

Atthia, H. H.: Preliminary experiments on the control of the two spotted spider mite on cotton. Agr. Research Rev. (Cairo) (Eng. ed.) **36**, 138 (1958); through Rev. Applied Entomol. (Ser. A) **47**, 413 (1959).

Bailey, J., and R. P. Curry: Geigy int. method FPTM-D-36-1 (1965).

Baker, E. A., and E. Skerret: Determination of DDT and chlorobenzilate occuring together in spray deposits. Analyst **83**, 447 (1958).

Baptist, B. A., and D. J. W. Ranaweera: The scarlet mites of the genus Brevipalpus as pest of tea in Ceylon. The Tea Quarterly **26** (IV), 127 (1955).

Bartlett, B. R.: The contact toxicity of some pesticide residues to hymenopterous parasites and coccinellid predators. J. Econ. Entomol. **56**, 694 (1963).

BASU, A. C., and L. M. PRAMANIK: Acaricidal tests of nine pesticides against the two-spotted spider mite, a serious pest of brinjal (eggplant) in West Bengal. J. Econ. Entomol. **61,** 768 (1968).

BECKMANN, H., and A. BEVENUE: Microcoulometric gas chromatographic analysis of grapes and cottonseed for chlorobenzilate residues. J. Agr. Food Chem. **12,** 183 (1964).

BENFIELD, C. A., and D. RICHARDSON: Internal reports CP/63/Anal/20, CP/64/Anal/21. Chesterford Park Research Station, Saffron Walden, Essex (1965).

BERAN, F.: Bienentoxizität und Bienengefährlichkeit von ROSPIN 25. Briefliche Mitteilung vom Oct. 13 (1969).

—, and J. NEURURER: Zur Kenntnis der Wirkung von Pflanzenschutzmitteln auf die Honigbiene (*Apis mellifera* L.). Pflanzenschutzberichte **XVII,** 113 (1956).

BEYE, F., W. KAESER, and R. BUCHNER: Zur Wirkung von Aktivsubstanzen verschiedener Insektizide auf Bienen. I. und II. Mitteilung. Anz. Schädlingskunde **32,** 41 and 121 (1959).

BLANCK, A., L. MIQUEL, and J. MOTEMPS: Lutte contre l'acariose de la tomate. Phytoma **9** (80), 37 (1956).

BLINN, R. C., F. A. GUNTHER, and M. J. KOLBEZEN: Microdetermination of the acaricide p,p'-dichlorobenzilate (Chlorobenzilate). J. Agr. Food Chem. **2,** 1980 (1954).

BRAKEY, E. F., and G. S. BATCHELOR: Biology and control of the dryberry mite *Phyllocoptes gracilis* (Nal.). Bull. Wash. Agr. Expt. Sta. No. 574 (1957); through Rev. Applied Entomol. (Ser. A) **47,** 224 (1959).

BULLOCK, R. C.: Citrus rust mite control. Agr. Aviation **7,** 114 (1965).

CABANE, E.: Mise au point sur les acariens et sur le nouvel acaricide G 24163. La Défense des Végétaux **123,** 79 (1967).

Canada Department of Agriculture (Ottawa), Pesticide Unit, Plant Products Division: Compendium on registered uses of pesticides in Canada (1969).

CARTER, R. H.: Estimation of DDT in milk by determination of organic chlorine. Anal. Chem. **19,** 54 (1947).

—, and P. E. HUBANKS: Determination of DDT deposits on fruits, vegetables, and vegetation. J. Assoc. Official Agr. Chemists **29,** 112 (1946).

—, R. H. NELSON, and A. GERSDORFF: Organic-chlorine determination as a measure of insecticide residues in agricultural products. Adv. Chem. Ser. **1,** 271 (1950).

CASSIDY, J. E., B. MIN, and R. T. MURPHY: Metabolic fate of C^{14}-chloropropylate in white rats. A balance study. Unpublished report, Analytical Research Department, Geigy Research Division, *Geigy Chemical Corp.* Ardsley, N. Y. (1968).

CHABOUSSOU, F.: Résultats d'essais acaricides vis à vis de *Bryobia praetiosa* Koch sur pêcher et de Metatetranychus ulmi Koch sur pommier en 1955. Phytiatrie Phytopharmacie **5,** 203 (1956).

—, and R. RAMADIER: La lutte contre les pucerons et les acariens des arbres fruitiers. Essai de nouveaux produits. Rev. Zool. Agric. **55,** 117 (1956); through Rev. Applied Entomol. (Ser. A) **46,** 139 (1958).

Chesterford Park Research Station (Nr. Saffron Walden, Essex): The toxicity of chloropropylate to fish. Unpublished report from Medical Department, *Fisons Pest Control Limited,* Report Tox/111/3 (1964).

CLEVELAND, M. L.: Field studies in the control of orchard mites in 1957. J. Econ. Entomol. **51,** 713 (1958).

CLOWER, D. F., and J. G. MATTHYSSE: Phytotoxicity of insecticides in mist concentrate type formulation. J. Econ. Entomol. **47,** 735 (1954).

COULSON, D. M.: Electrolytic conductivity detector for gas chromatography. J. Gas Chromatog. **3,** 134 (1965).

— Selective detection of nitrogen compounds in electrolytic conductivity gas chromatography. J. Gas Chromatog. **4,** 285 (1966).

—, L. A. CAVANACH, S. E. DE VRIES, and B. WALTHER: Microcoulometric gas chromatography of pesticides. J. Agr. Food Chem. **8,** 399 (1960).

CUMMINGS, J. R.: Pesticides in the total diet. Residue Reviews **16,** 30 (1966).

DAVIS, B., J. HENDRIX, and R. P. GIDEON: Geigy int. method FPTM-D-36-5 (1968).

DELLEY, R.: Geigy int. method ROS Nr. 1272 (1957).
—, K. FRIEDRICH, and A. GEISER: Geigy int. method ROS Nr. 2526 (1964).
DOMENJOZ, R.: Chlorobenzilate — Toxicity in chronic administration. Unpublished report, Institute of Pharmacology, *Rheinische Friedrich-Wilhelms University*, Bonn, Ger. (1965 a).
— Dichlorobenzilic acid — Toxicity in chronic administration. Unpublished report, Institute of Pharmacology, *Rheinische Friedrich-Wilhelms University*, Bonn, Ger. (1965 b).
DOWNING, R. S.: Recent trials with new acaricides in British Columbia orchards. Canad. J. Pl. Sci. **38**, 61 (1958); through Rev. Appl. Entomol. (Ser. A) **47**, 124 (1959).
DREHER, K., H. HIRSCHFELDER, and H. SACHS: Die Heilkur gegen die Milbenseuche der Bienen mit Folbex Räucherstreifen. Südwestdeutscher Imker. S., p. 42 (1958).
DURHAM, W. F.: The interaction of pesticides with other factors. Residue Reviews **18, 21** (1967).
EBELING, W., and R. J. PENCE: Susceptibility to acaricides of two-spotted spider mites in the egg, larval and adult stages. J. Econ. Entomol. **47**, 789 (1954).
ELMER, H. S., and L. R. JEPPSON: Biology and control of the citrus flat mite. J. Econ. Entomol. **50**, 566 (1957).
FAO/WHO: 1968 Evaluation of some pesticide residues in food, pp. 42–68. Rome (1969).
Federal Register (U.S.A.) **27**, F.R. 12902 (1962).
Food and Agriculture Organization of the United Nations: Pesticide residues in food. Report 1968 Joint FAO/WHO Meeting, Rome (1969).
FURR, R. E., and L. B. DAVIS: Acaricides for control of the two-spotted spider mite on cotton in the field. J. Econ. Entomol. **62**, 732 (1969).
GASSER, R.: Über zwei neue Akarizide aus der Gruppe der Di-(p-chlorphenyl)-karbinole. Experientia **8**, 65 (1952).
— Le problème des araignées rouges en culture fruitière. Congrès pomologique Internat. **86**, 135 (1955).
— Uses of pesticides in selective manners. Proc. FAO Symposium on Integrated Pest Control **2**, 109 (1966).
—, and H. GROB: The acaricidal activity of some esters of dichlorobenzilic acid. Second British Insecticide and Fungicide Conference, Brighton, pp. 4–7 and 411–419, Nov. (1963).
Geigy Chemicals Corp., Ardsley, N. Y., U.S.A: Residue reports. Unpublished information (1970).
Geigy, J. R. — S. A., Basel, Switzerland: The toxicology of chlorobenzilate. Information Bull. (1954).
— Chloropropylat im Vergleich zu Chlorbenzilat. Information aus der Abt. für Schädlingsbekämpfung, Ref. 72/HAE/18. 2. (1955).
— Chlorpropylat, Akute Toxizität — Maus per os. Unpublished report, Vers. Nr. 12017, 17. 8. (1962 a).
— Chlorpropylat, Akute Toxizität — Ratte per os. Unpublished report, Vers. Nr. 12018, 17. 8. (1962 b).
— Chlorpropylat, Subchronische Toxizität — Ratte per os. Unpublished report, Laboratories for Experimental Toxicology and Laboratories for Experimental Pathology (1962 c).
— Chlorpropylat (G 24163), Subchronische Toxizität — Ratte (Fütterungsversuche: 12 Wochen). Unpublished report, Laboratories for Experimental Toxicology and Laboratories for Experimental Pathology (1963).
— Chloropropylate (G 24163). Ref. Pesticide Research Department 3/WB/hh, 30. 1. (1964 a).
— Toxicology of Chloropropylate (G 24163). 2nd communication. Information Bull. (1964 b).
— Chlorobenzilate (New Edition 1965). Information issued by Pest Control Department. Ref. 72/HS/RT/15. 2. (1965).
— Residue reports. Unpublished information (1970).

GUBLER, H. U., A. BRUEGGER, H. SCHNEIDER, R. GASSER, and R. WYNIGER: Über ein neues spezifisches Mittel zur Bekämpfung der Bienenmilben. Schweiz. Bienenzeitung **76**, 268 (1953).

GUNTHER, F. A., L. R. JEPPSON, and G. B. WACKER: Persistance of Chlorobenzilate residues in mature lemon fruits. J. Econ. Entomol. **48**, 372 (1955).

HARRIS, H. J.: Colometric determination of ethyl 4,4′-dichlorobenzilate (Chlorobenzilate) as a spray residue. J. Agr. Food Chem. **3**, 939 (1955).

HASSAN, T. K., and CH. O. KNOWLES: Behaviour of three C^{14}-labeled benzilate acaricides when applied topically to soybean leaves. J. Econ. Entomol. **62**, 618 (1969).

Hazleton Laboratories, Inc., Falls Church, Va., U.S.A.: Geigy 338 technical. Feeding studies — Rats. Unpublished report (1953 a).

— Geigy 338 technical. Analysis of animal tissue for Geigy 338. Unpublished report (1953 b).

— Geigy 338, technical Chlorobenzilate. Chronic feeding — Rats. Analysis of animal tissue for Geigy 338. Unpublished report (1953 c).

— Chlorobenzilate and chloropropylate. Method development for the analysis of chloropropylate and chlorobenzilate in biological fluids and tissue specimens. Metabolic distribution and excretion of chloropropylate and chlorobenzilate in dogs. Unpublished report (1964).

— Chlorobenzilate, two-year dietary feeding study — Dogs. Final report. Unpublished report (1965 a).

— Chloropropylate 50 W — Three-month dietary administration — Dogs. Final report. Unpublished report (1965 b).

HENDRIX, J.: Geigy int. method FPTM-D-36-3 (1969).

Hill Top Research Institute, Inc., Miamiville, Ohio, U.S.A.: Repeated insult patch test on Chlorobenzilate 50 E and technical chlorobenzilate. Unpublished report (1963).

HOPP, H.-H.: Beiträge zur Wirkungsweise einiger Akarizide auf Eier der Spinnmilben *Paratetranychus pilosus*, Can. et Franz. und *Tetranychus urticae* Koch. Z. ang. Zoologie **41**, 269 (1954).

HORN, H. J., R. B. BRUCE, and O. E. PAYNTER: Toxicology of chlorobenzilate. J. Agr. Food Chem. **3**, 752 (1955).

HOWITT, A. J., and A. PSHEA: The development and use of ultra low volume ground sprayers for pests attacking fruit. Quart. Bull. Mich. Agr. Expt. Station, *Michigan State University*, East Lansing **48** (2), 144 (1960).

Industrial Biology Laboratories, Inc.: Technical Chloropropylate. Repeated insult patch test. Unpublished report (1965).

Industrial Bio-Test Laboratories, Inc., Northbrook, Ill., U.S.A.: Acute toxicity studies: Chlorobenzilate 25 W. Unpublished report (1965 a).

— Acute toxicity studies: Chlorobenzilate 4 E. Unpublished report (1965 b).

— 21-Day subacute dermal toxicity of Chlorobenzilate 25 W. Unpublished report (1965 c).

— Chloropropylate 25 E: Acute toxicity studies. Unpublished report (1965 d).

— Chloropropylate 40 W: Acute toxicity studies. Unpublished report (1965 e).

— 21-Day subacute dermal toxicity of Chloropropylate 40 W. Unpublished report (1966).

— 21-Day subacute dermal toxicity of Chlorobenzilate 4 E. Unpublished report IBT No. A 4646 (1967 a).

— 21-Day subacute dermal toxicity of Chloropropylate 25 E. Unpublished report IBT No. A 4647 (1967 b).

— Two-year chronic oral toxicity of Chloropropylate 40 W: Beagle dogs. Unpublished report IBT No. C 4645 (1968).

JEFFERSON, R. N., and F. S. MORISHITA: Azobenzene-aramite and azobenzene-chlorobenzilate sprays for resistent spider mites on greenhouse roses. J. Econ. Entomol. **49**, 151 (1958).

JEPPSON, L. R.: New acaricide for citrus mites. Calif. Agr. **9** (6), 11 (1955 a).

—, M. J. JESSER, and J. R. COMPLIN: Control of mites on citrus with Chlorobenzilate. J. Econ. Entomol. **48**, 375 (1955 b).

— — — Effectiveness of two new phosphate insecticides for control of mite injurious to citrus in California. J. Econ. Entomol. **50**, 307 (1957 a).

JEPPSON, L. R., H. S. ELMER, M. J. JESSER, and J. O. COMPLIN: Effectiveness of 4,4'-dichloro-alpha(trichloromethyl)benzhydrol(F-293) for control of citrus mites in California. J. Agr. Food Chem. **5**, 592 (1957 b).

—, M. J. JESSER, and J. O. COMPLIN: Factors affecting populations of the citrus bud mite in southern California lemon orchards and acaricide treatments for control of the eriophyd. J. Econ. Entomol. **51**, 657 (1958).

JOHANSEN, C. A.: Digest on bee poisoning, its effects and prevention with an annotated list of 92 insecticides. Bee World **47** (1), 9 (1966).

LARSEN, M., E. LIFART, and R. VAN CAUWENBERGHE: Geigy int. method Nr. S 444 (1970).

LEIGH, T. F.: Control of spider mites on cotton very necessary in arid regions. Cotton Trade J. and Agr. Reporter Memphis **42** (20) (1962).

—, C. E. JACKSON, V. R. BURTON, and J. H. BLACK: Acaricides for tetranychid mite control of cotton. J. Econ. Entomol. **60**, 718 (1967).

LE PELLEY, R.: The citrus bud mite, *Aceria sheldoni* (Ewing) Eriophyidae in Kenya. E. Afr. Agr. J. **21**, 22 (1955); through Rev. Applied Entomol. (Ser. A) **44**, 211 (1956).

MARGOT, A., and K. STAMMBACH: Chlorobenzilate. In ZWEIG, G. (ed.): Analytical methods for pesticides, plant growth regulators, and food additives, vol. **2**, pp. 65–73. New York-London: Academic Press (1964).

MATTSON, A. M., and R. KAHRS: Geigy int. method No. AG 13 (1962).

—, R. L. BEAUDOIN, and J. SCHNELLER: Comparison of urinary metabolites in dogs after administration of chlorobenzilate or chloropropylate. Unpublished report, Analytical Department, Geigy Research Laboratories, Division of *Geigy Chemical Corp.*, Ardsley, N. Y. (1965).

—, M. INSLER, and J. SCHNELLER: Chlorobenzilate residues in sheep and cattle tissues. Unpublished report, Analytical Department, Geigy Research Laboratories, Division of *Geigy Chemical Corp.*, Ardsley, N. Y. (1966).

—, T. CULLEN, and R. KAHRS: Residue of chlorobenzilate and its degradation products in dried tea. *Geigy Chemical Corp.*, Ardsley, N. Y. Analytical Research Department. Unpublished report no. GAAC 68012 (1970).

MEYER, R., and R. SUTER: Geigy int. method ROS No. 495 (1953).

MISTRIC, W. J.: Control of spider mites on cotton. J. Econ. Entomol. **57**, 855 (1964).

MIYAZAKI, S., G. M. BOUSH, and F. MATSUMURA: Microbial degradation of chlorobenzilate (ethyl 4,4'-dichlorobenzilate) and chloropropylate (isopropyl 4,4'-dichlorobenzilate). J. Agr. Food Chem. **18**, 87 (1970).

MORISHITA, F. S.: Biology and control of *Brevipalpus inornatus* (Banks). J. Econ. Entomol. **47**, 449 (1954).

MULLER, E. W.: Untersuchungen über den Einfluß chemischer Pflanzenschutzmittel auf den Populationsverlauf von Spinnmilben und Raubmilben im Obstbau. Nachrichtenbl. Deutsch. Pflanzenschutzd. (Berlin) **14**, 221 (1960).

MURPHY, R., R. KAHRS, and A. M. MATTSON: Dissipation of residues of chlorobenzilate and chloropropylate on apples. Analytical Department, *Geigy Chemical Corp.*, Ardsley, N. Y. Unpublished report (1966).

OATMANN, E. R.: European red mite control and population studies in Wisconsin. J. Econ. Entomol. **52**, 871 (1959).

RAMBIER, A.: Les tetranyques nuisibles à la vigne en France continentale. Rev. Zool. agric. **57**, 1 (1958); through Rev. Applied Entomol. (Series A) **48**, 108 (1960).

RANAWEERA, D. J. W.: Acaricidal trials against the scarlet mite, *Brevipalpus australis* Tucker on tea. Tea Quarterly **29**, 125 (1958); through Rev. Applied Entomol. (Serie A) **48**, 534 (1960).

REED, C. R., D. K. CRITTENDEN, and D. J. LYON: Acaricides screened against two rust mites of citrus. J. Econ. Entomol. **60**, 668 (1967).

ROTH, F. J.: A one-shot, two-filter application of the Schechter method for mixed DDT and chlorobenzilate spray residues. J. Assoc. Official Agr. Chemists **41**, 662 (1958).

SANDERSON, D. M.: The avian toxicity of chloropropylate. Unpublished report, *Chesterford Park Research Station*, Medical Dept. *Fisons Pest Control Limited*, Saffron Walden, Essex. Report Tox/111 (1963).

SCHAEFERS, G. A.: Control tests against the two-spotted spider mite, *Tetranychus telarius* (L.), on strawberries. J. Econ. Entomol. **58,** 1089 (1965).

SCHECHTER, M. S., S. B. SOLOWAY, R. A. MAYES, and H. L. HALLER: Colorimetric determination of DDT. Ind. Eng. Chem., Anal. ed. **17,** 704 (1945).

SCHNEIDER, H.: Die Milbenbekämpfung mit Folbex. Schweiz. Bienenzeitung **82,** 105 and 156 (1959).

SCHWARTZ, A., and F. J. RIEKERT: Effectiveness of standard and new compounds for the control of bud mite, *Aceria sheldoni* Ewing on naval oranges. S. Afr. J. Agr. Sci. **10,** 609 (1967).

STEPANOW, A.: Über die Halogenbestimmung in organischen Verbindungen mittels metallischen Natriums und Äthylalkohols. Ber. **39,** 4056 (1906).

STERNLICHT, M.: Further trials in the control of the citrus bud mite *Aceria sheldoni* Ewing (Eriophyidae, Acarina). Z. ang. Entomol. **64,** 135 (1969).

SUTER, R., K. FRIEDRICH, and G. SZEKELY: Geigy int. method ROS No. 832 (1963).

SWIRSKI, E., S. AMITAL, and N. DORZIA: Field and laboratory trials on the toxicity of some pesticides to predaceous mites (Acarina: Phytoseiidae). Israel J. Agr. Research **17,** 149 (1967).

Tocklai Experimental Station, Assam (India): Tea Research Association: Annual scientific report 1964, pp. 21, 22, 75, 80, and 84 (1965).

UMHOEFER, R. R.: Determination of halogens in organic compounds. Ind. Eng. Chem., Anal. ed. **15,** 383 (1943).

WESTIGARD, P. H., and D. W. BERRY: Control of the pear rust mite. *Epitrimerus pyri.* J. Econ. Entomol. **57,** 953 (1964).

WOLFE, H. R.: Acaricides in insect vector virus research. J. Econ. Entomol. **48,** 749 (1955).

Woodard Research Corp., Herndon, Va., U.S.A.: Examination of milk for residues following feeding of chlorobenzilate to dairy cows. Unpublished report (1960).

— Safety evaluation and determination of the amount of chlorobenzilate that might appear in the tissues of sheep and beef cattle fed this material in the diet for four weeks. Unpublished report (1965 a).

— Chlorobenzilate — Three-generation reproduction study in the rat. Unpublished report (1965 b).

— Chlorobenzilate — Safety evaluation on fish and wildlife (bobwhite quail, mallard ducks, rainbow trout, sunfish, goldfish, oysters). Unpublished report (1965 c).

— Chlorobenzilate 25 W. The effect on shell growth in oysters. Unpublished report (1966 a).

— Chlorobenzilate — Safety evaluation by dietary feeding to rats for 104 weeks — Final report. Unpublished report (1966 b).

— Chloropropylate — Safety evaluation on fish and wildlife (bobwhite quail, rainbow trout, bluegill sunfish, and goldfish). Unpublished report (1966 c).

— Chloropropylate — Subacute toxicity in mallard ducks. Unpublished report (1966 d).

— Chloropropylate — The effect on shell growth in oysters. Unpublished report (1966 e).

— Chloropropylate — Safety evaluation by dietary feeding to rats for 104 weeks — Final report. Unpublished report (1966 f).

— Chloropropylate — Three-generation reproduction study in the rat. Unpublished report (1966 g).

— Chloropropylate — Histopathological report addenda. Unpublished report (1967).

World Health Organization: Technical report series no. 417 (Geneva) (1969).

WURZSCHMITT, B.: Ein neues Schnellaufschlußverfahhren mit Alkaliperoxid mit einer Universalbombe für Mikro-, Halbmikro-Makroeinwaagen. Chem. Ztg. **74,** 356 (1950 a).

— Die Metallbombe als Hilfsmittel in der Elementaranalyse. Fortschr. chem. Forsch. **1,** 485 (1950.b).

— Ein neues Schnellaufschlußverfahren mit Alkaliperoxid in einer Universalbombe für Mikro-, Halbmikro- und Makroeinwaagen. Mikrochem. ver. Mikrochim. Acta **37,** 769 (1951).

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