Stability of Isopropyl *N*-Phenylcarbamate (IPC) and Isopropyl *N*-(3-Chlorophenyl)-carbamate (CIPC) Residues on Fruit Treated after Harvest

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The stability of isopropyl *N*-phenylcarbamate (IPC) and isopropyl *N*-(3-chlorophenyl)-carbamate (CIPC) was studied when applied to fruits after harvest to extend storage life. The residues were determined by a colorimetric method comprising extraction of the residues with benzene, hydrolyzing IPC and CIPC with sulfuric acid, and coupling the diazodized aniline or chloroaniline with *N*-(1-naphthyl)ethylenediamine into an azo dye which was measured at 555 mμ. The fruit was treated after harvest by dipping in emulsions of the chemicals. The correlations between initial deposits and dip concentrations or dipping time were studied. In all the trials, IPC and CIPC behaved similarly, producing very stable residues on tomatoes, plums, and apples. The losses on apples at 0° C. were about 30% in 5 months and at 10° C. about 50% in 3 months. In preservation processes the losses of IPC and CIPC were always less than 50%. Incubation of CIPC in the homogenates of tomato fruits and spinach leaves did not reveal any enzymatic degradation of the chemical. Washing treated fruits with water resulted in about 25% losses just after treatment, whereas later washings removed very little if any residues.

THE aryl carbamates, isopropyl *N*-phenylcarbamate (IPC) and isopropyl *N*-(3-chlorophenyl)carbamate (CIPC), are primarily used as herbicides (*1*) and sprout inhibitors of potato tubers (*21*). Mukula (*19*) demonstrated that IPC and CIPC prevented the growth of several fungal pathogens in carrots on artificial culture media, but their inhibitory effects were less pronounced on stored carrots. Smoot *et al.* (*22*) found that these chemicals were promising decay inhibitors for postharvest use on Florida oranges and could probably be effectively used on other fruits and vegetables. The authors of the present paper have determined (*14*) that IPC and CIPC applied after harvest to certain fruits—e.g., apples, plums, and tomatoes—may extend their storage life by decreasing fungal decay and retarding the development of physiological disorders, but on some other products the effect may be reversed.

The determination and amounts of IPC and CIPC residues on field crops (*3, 7–11, 16, 17*) and on stored potatoes (*5, 6, 20*) have been reported. These results indicated that if IPC and CIPC are properly used, residues on food commodities are very low.

Materials and Methods

Extraction. The IPC and CIPC residues were extracted by the method used for malathion postharvest residues (*13*). Half a kilogram of unmacerated plant material was tumbled with 0.5 liter of benzene for 1 hour, after which the extract was dried with anhydrous Na_2SO_4 without other cleanup treatment. The plant material was not macerated prior to extraction, since preliminary trials on tomatoes and two varieties of apples definitely showed that maceration did not increase the recoveries of even 5-month-old residues, and recoveries were not increased by extracting the fruits after maceration with benzene or with benzene-ethanol. At least two extractions were made from each material to be analyzed.

Analytical Method. After encountering difficulties with the reproducibility (cf. *16*) of the analytical method for IPC and CIPC residues described by Gard *et al.* (*7–10*), a colorimetric method was evolved, based on the color reaction of the diazodized aniline or 3-chloroaniline with *N*-(1-naphthyl)ethylenediamine. This procedure, derived from the method for parathion residues (*2, 15*), is also used by other workers (*6, 11, 18*) for phenylcarbamate residues, but in slightly different forms.

For hydrolyzing IPC or CIPC, 10 ml. of benzene extract containing less than 200 μg. of the compound to be analyzed was transferred with 20 ml. of 1 to 1 dilute sulfuric acid and two glass beads to a 500-ml. round-bottomed flask provided on a ground joint with a 30-cm. long Allihn condenser. The mixture was allowed to boil and reflux slowly for 1 hour on an electric mantle. After cooling for 10 minutes, 80 ml. of distilled water was added through the condenser. The benzene was removed from the solution by evaporation at 40° C. on a water bath and at reduced pressure. After evaporation, 3 drops of Antifoam RD (Dow Corning Co., Midland, Mich.) was added to the flask to retard foaming during steam distillation of the aniline and chloroaniline. The flask was then connected to the distillation apparatus. The outlet tube of the condenser was immersed in a 50-ml. volumetric cylinder containing 5 ml. of 1.7*N* HCl and kept cool in crushed ice. Fifty milliliters of 50% NaOH was introduced cautiously into the flask, heat was applied, and the distillation was conducted until about 40 ml. of the distillate was collected. After collection, the distillate was transferred quantitatively to a 50-ml. volumetric flask. One milliliter of 0.25% $NaNO_2$ solution was added as a diazodizing agent, and the flask was shaken for 15 seconds and allowed to stand for 10 minutes. For removing the excess $NaNO_2$, 1 ml. of 2.5% ammonium sulfamate was added and the flask was shaken for 15 seconds and allowed to stand for 10 minutes. Color was developed by adding 2 ml. of 1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution, and the flask was filled to the mark with 0.2*N* HCl, shaken for 15 seconds, and allowed to stand until maximum color intensity was attained. The solution was filtered through cotton into a 1- or 5-cm. cuvette and its absorbance measured with a Beckman DU spectrophotometer at 555 mμ against distilled water.

At least two replicate determinations were made from each benzene extract. The blank absorbance values recorded from untreated plant materials were 0.015 to 0.035 for the 1-cm. cell. If an absorbance reading twice as high as that of the blank is considered to be the sensitivity limit of the method, amounts of IPC and CIPC as small as 10 μg. can

be measured by the procedure described.

For preparing standard curves, pure CIPC (N. V. Fabriek van Chemische Producten, Vondelingenplaat, Holland) and 98.0% IPC (FMC International, Ltd., New York, N. Y.) were used. The absorption maxima of the red dyes formed were 558 mμ for aniline and 548 mμ for 3-chloroaniline. The maximum color intensity for aniline developed in $1^1/_2$ hours and for chloroaniline in 10 minutes; the colors are very stable.

To test the reliability of the analytical method, the extraction benzene was fortified with known amounts of IPC and CIPC before tumbling. The recoveries are shown in Table I.

Dipping as Application Method. To test dipping as the application method for IPC and CIPC, 1-kg. portions of fruits were immersed either for a fixed time in different concentrations or for varied times in the same concentration (cf. *13*). Dip solutions were prepared from 40% emulsifiable concentrates (40% by weight 98% IPC or CIPC, 8% Triton X-100, and 52% xylol). The fruits tested were: strawberries (var. Ydun), average weight 4.2 grams; gooseberries (var. Houghton), average weight 1.1 grams; apples (var. Chanel), average weight 52 grams; plums (var. Victoria), average weight 30 grams; and tomatoes (var. Grower's Pride), average weight 63 grams.

The results appear in Figure 1 for dip concentrations and in Figure 2 for dipping times. The chemicals behaved similarly in respect to their ability to form residues on the fruits. The residues were much higher on strawberries, gooseberries, and apples than on plums and tomatoes, a variation which can be attributed to differences in the nature of the fruit surfaces. When the amounts of residues were calculated per unit of surface area, the lowest values were obtained for tomatoes—e.g., 25 μg. per sq. cm. with the 0.1% dip for 30 seconds—on plums the figures were about $1^1/_2$ times, on gooseberries 3 to 8 times, on apples 5 to 10 times, and on strawberries more than 10 times higher. The correlation between the initial deposit and the dip concentration above 0.1% seems to be nearly linear (Figure 1). Increasing the dipping time from 5 seconds to 2 to 3 minutes caused a greater increase in deposits than when longer dipping times were used (Figure 2). When comparing these figures with those obtained from malathion emulsions on corresponding plant materials (*13*), the most outstanding observation is that IPC and CIPC gave much higher residues than malathion.

Table I. Recoveries of IPC and CIPC from Fortified Samples

Material	Added, P.P.M.	Recovered, %[a] IPC	CIPC
Cabbage	0.5	97	105
	2.0	86	93
	5.0	92	98
	40.0	91	104
	200.0	92	97
New Zealand spinach	0.5	120	90
	2.0	98	98
	5.0	95	104
	40.0	102	112
	200.0	97	96
Tomato fruits (ripe)	0.5	...	100
	2.0	...	98
	5.0	...	103
	40.0	...	110
	200.0	...	91
Tomato fruits (green)	0.5	102	88
	2.0	107	88
	5.0	98	86
	40.0	95	86
	200.0	96	105
Strawberry fruits	2.0	...	90
	4.0	...	110
	40.0	...	94
	200.0	...	113

[a] Mean value; two determinations on duplicate samples for each concentration.

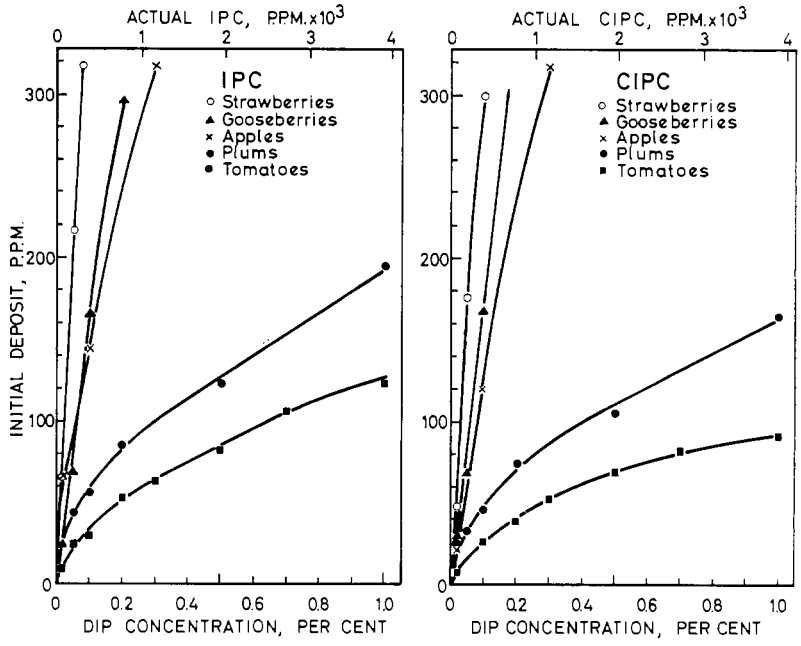

Figure 1. Effect of concentrations of dip emulsions on initial depostis of IPC and CIPC

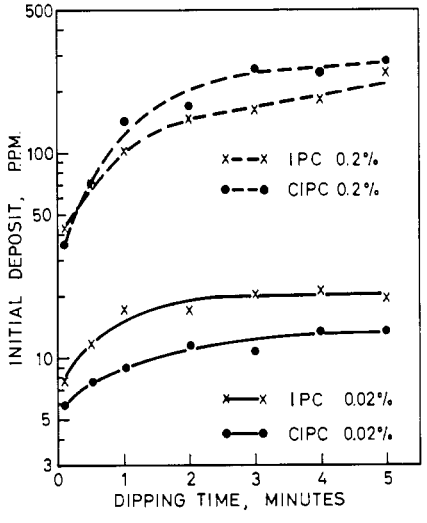

Figure 2. Effect of dipping time on amount of IPC and CIPC initial deposits from emulsions of two concentrations on tomatoes

Disappearance of Residues from Stored Fruits

The disappearance of IPC and CIPC residues from fresh fruits treated after harvest was studied on tomatoes (var. Grower's Pride), plums (var. Victoria), and apples (var. Åkerö). The fruits were treated by dipping in an IPC or CIPC emulsion of a specified concentration to produce a residue of around 50 p.p.m. and then stored at different temperatures. Samples of each batch were analyzed for the residues at the beginning of storage and at later intervals.

The results (Table II) show that the disappearance rates of IPC and CIPC were about the same under the same circumstances. Increasing the storage temperature generally enhanced the rate of disappearance.

Residue Losses from Preservation

Gooseberry Processing. Gooseberries (var. Houghton) were treated after harvest by dipping in a 0.01% emulsion of IPC or CIPC made from the 40% concentrates. One day after treatment the berries, having residues of 5 to 10 p.p.m., were canned or made into jam.

Canning. Berries (500 grams) and 40% sugar solution (200 ml.) were

Table II. IPC and CIPC Residues[a] on Fruits Treated with Pesticide Emulsion[b] after Harvest and Stored at Different Temperatures

Time, Weeks	0° C. Tomatoes P.p.m.	0° C. Tomatoes Loss, %	0° C. Plums P.p.m.	0° C. Plums Loss, %	0° C. Apples P.p.m.	0° C. Apples Loss, %	10° C. Tomatoes P.p.m.	10° C. Tomatoes Loss, %	10° C. Plums P.p.m.	10° C. Plums Loss, %	10° C. Apples P.p.m.	10° C. Apples Loss, %	20° C., Tomatoes P.p.m.	20° C., Tomatoes Loss, %
						IPC								
0	57.1	0	43.4	0	16.4	0	70.9	0	43.4	0	16.4	0	57.1	0
1	56.8	1	38.5	11	...	...	...	...	34.5	21	...	...	53.2	7
2	57.1	0	38.5	11	...	...	62.8	12	33.4	23	...	...	33.8	41
3	45.8	20	...	...	...	...	...	...	33.4	23	...	...	28.4	50
4	50.4	12	38.2	12	...	...	56.6	20	...	...	...	...	21.4	63
6	...	...	31.6	27	...	...	50.4	29	...	...	...	...	...	...
8	...	...	...	...	12.5	24	...	...	...	...	14.3	13	...	...
12	...	...	...	...	...	...	...	...	...	...	7.8	52	...	...
20	...	...	...	...	11.8	28	...	...	...	...	...	...	...	...
						CIPC								
0	58.4	0	61.5	0	29.2	0	70.0	0	61.5	0	29.2	0	58.4	0
1	54.6	7	47.8	22	...	...	...	...	59.9	3	...	...	54.2	7
2	52.0	11	41.0	33	...	...	56.5	19	42.1	32	...	...	47.3	19
3	54.7	6	...	...	...	...	...	...	38.7	37	...	...	28.9	51
4	53.1	9	45.9	25	...	...	49.6	29	...	...	...	...	25.7	56
6	...	...	44.1	28	...	...	45.4	35	...	...	...	...	...	...
8	...	...	...	...	21.1	28	...	...	...	...	18.4	37	...	...
12	...	...	...	...	...	...	...	...	...	...	15.2	48	...	...
20	...	...	...	...	19.5	33	...	...	...	...	...	...	...	...

[a] Each figure is mean of two determinations from three or four 0.5-kg. samples.
[b] Dip concentrations. Tomatoes 0.2%, plums 0.1%, apples 0.02%; dipping time 30 seconds.

placed in glass jars and autoclaved for 20 minutes at 120° C. and the product was allowed to cool to the room temperature.

JAM. Berries and 70% sugar solution (1 to 0.85, by weight) were boiled for 20 minutes in an aluminum kettle, and the product was put into glass jars, and allowed to cool to room temperature.

The preservation processes were replicated several times, and the total losses of IPC and CIPC were found to vary considerably. In canning, the losses of IPC ranged from 8 to 31% (average 21%), and of CIPC from 27 to 43% (average 32%). In jam making, IPC losses were 11 to 28% (average 18%) and CIPC losses 9 to 37% (average 26%). These figures indicate that CIPC residues disappeared in preservation processes slightly more rapidly than IPC residues and that both chemicals have a stable molecular structure.

Autoclaving in Buffer Solution. To find out whether the plant material had an effect on the stability of IPC and CIPC during autoclaving, 30 mg. of each of these compounds was added to a liquid mixture consisting of 290 ml. of McIlvaine's phosphate-citric acid buffer solution (pH 4 or 6) and 10 ml. of ethanol. The mixture was autoclaved for 1/2 hour in closed jars at 120° C.

In all cases the results were very similar, showing a loss of 16% for both IPC and CIPC at both pH values. Comparing these figures with those from the preservation trials (losses of about 20 to 25%), it can be assumed that during autoclaving the plant material slightly catalyzed the degradation of IPC and CIPC or that these chemicals were partially bound in an inextractable form.

Rate of Hydrolysis. Since the previous experiments indicated that IPC and CIPC are very stable, their rate of hydrolysis was measured in a solution containing 240 ml. of McIlvaine's phosphate–citric acid buffer solutions, 60 ml. of absolute ethanol, and 40 p.p.m. of CIPC or IPC at pH 1 and 9 at 70° C. The rate of hydrolysis was followed by analyzing duplicate samples at the beginning of the experiment and at certain periods of time thereafter. The half life of hydrolysis for both chemicals and at both pH values was 6 months. This long period indicates that spontaneous degradation of IPC and CIPC cannot play an appreciable role in their disappearance from plants, soils, or food commodities, and even cooking or other heat-processing methods have only a very limited effect in hastening their degradation.

Washing. Since raw plant materials are often washed before preserving, trials were carried out to determine the possible losses of IPC and CIPC residues caused by this process. Tomatoes and plums were dipped in an IPC or CIPC emulsion after harvest and subsequently washed for 1 minute in a strainer under running tap water.

Table III shows that a few hours after treatment 1/5 to 1/4 of the residues was removed by the washing. After 1 week, however, and thereafter practically none of the chemicals were removed. This indicates that the residues of ICP and CIPC are very firmly bound in the plant surface, probably dissolved in the lipids of the cuticle.

Disappearance from Plant Homogenates

The disappearance of CIPC from homogenized plant materials was investigated with tomato fruits (var. Grower's Pride) and New Zealand spinach leaves (cf. *12*).

The plant materials were homogenized at room temperature in McIlvaine's phosphate–citric acid buffer solution (ratio 2 to 1 w./v.). The pH of the buffer solution was the same as that of the plant material: 4.2 for tomatoes and 5.8 for spinach. Three different batches of material were used: fresh homogenates, homogenates heated at

Table III. Effect of Washing on IPC and CIPC Residues

Age of Residue, Weeks	Residue, P.P.M. Before washing	Residue, P.P.M. After washing	Loss of Residue, %	Residue, P.P.M. Before washing	Residue, P.P.M. After washing	Loss of Residue, %
			Tomatoes			
0	57.1	44.1	22	58.4	43.8	25
1	56.8	53.4	6	54.6	54.9	0
3	45.8	44.9	2	54.7	55.0	0
			Plums			
6	31.6	29.8	6	44.1	42.3	4

Table IV. Losses of CIPC from Plant Homogenates

Incubation Time, Hours	Loss, %					
	Tomato			Spinach		
	Fresh	Thymol	Heated	Fresh	Thymol	Heated
2	3	0	6	3	0	+4
5	6	10	16	...	...	...
6	...	...	...	+2	9	7
8	15	18	5	...	...	...
24	14	30	25	+6	7	6
48	...	...	...	7	6	8

85° to 90° C. for 10 minutes to destroy enzymes, and homogenates to which 0.1% thymol was added to prevent bacterial activity (*4*).

The homogenates were made to contain 20 p.p.m. of CIPC. Homogenates were thoroughly mixed and two 150-gram samples were taken from each batch. Incubation was carried out at 37° C., and at certain intervals two replicate subsamples were analyzed for CIPC. The pH was measured at the end of the trial and found not to have changed.

The results (Table IV) did not definitely indicate enzymatic degradation of CIPC in the plant homogenates. The CIPC losses in all three batches of tomato homogenates, however, indicated that in this plant material small amounts of CIPC were probably nonenzymatically decomposed or bound in the plant constituents.

Conclusions

An analytical method was developed for determining IPC and CIPC residues on plant materials treated after harvest. The accuracy and reproducibility of the method were satisfactory (Table I).

Dipping as a postharvest application method of IPC and CIPC to several fruits was tested. Very high residues were produced (Figures 1 and 2), especially on strawberries, gooseberries, and plums (Figures 1 and 2). The type of fruit greatly affected the magnitude of the deposits.

When tomatoes, plums, and apples were treated after harvest with IPC and CIPC and then stored at 0° to 20°C., the residues were very stable (Table II). This indicates that evaporation of these chemicals from fruit surfaces maintained within this temperature range is very slow and that there is no effective mechanism in the tissue of these harvested fruits for degrading IPC and CIPC. The latter point of view was further supported by the absence of enzymatic degradation of CIPC in the homogenates of tomatoes and spinach in 1 to 2 days (Table IV). Moreover, the spontaneous hydrolysis of both chemicals was found to be so slow that it cannot play an important role in the disappearance of the residues from fruits. All these observations explain the high stability of IPC and CIPC postharvest residues.

The above points also account for the relatively small losses of IPC and CIPC when treated gooseberries were canned and made into jam. Washing tomatoes and plums just after chemical treatment reduced the residues by about one fourth, but later practically nothing was removed by running water. This fact, together with the high residues formed in dip treatments, indicates that IPC and CIPC are readily absorbed into the fruit skin, in which they are able to resist different degradative mechanisms. Most probably they do not migrate deep into the fruit flesh, since residues even several months old were completely extracted by stripping the surface with benzene.

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