Metabolic Fate of P^{32}-Labeled Dimethoate in Olive Fruits and Some Toxicological Implications

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Dimethoate was studied for use in the control of the olive fly and proved to be both effective and safe. Metabolism of P^{32}-labeled dimethoate in olives for eating and in olives yielding oil is very similar. In these fruits, dimethoate undergoes oxidation to the oxygen analog (P=O derivative) and hydrolysis to degradation products such as phosphoric acid or methylphosphoric acid or both. When dimethoate is applied to olives for oil, according to the recommended schedule, the oil is practically free from toxic residues. When dimethoate is applied to eating olives, the usual industrial process with NaOH produces further degradation and a strong extraction of the P-containing insecticidal residues.

THE WIDESPREAD USE of dimethoate [*O,O* - dimethyl *S* - (*N* - methylcarbamoylmethyl) phosphorodithioate] (Rogor) is related to the exceptional systemic activity of this material and to its low toxicity to mammals. Extensive experimentation has demonstrated the highly effective persistent activity of dimethoate against the olive fruit fly (*Dacus oleae* Gmel.). However, numerous microanalytical determinations using sensitive chemical methods and bioassays (*3, 4*) have established (*1, 2, 10, 11, 15*) the practical absence (*14*) of toxic residues in the oil obtained from olives. Further research (*13*) has provided an explanation of why olive oil is obtained without toxic residues in spite of exceptional persistent insecticidal activity of the active ingredient, and has indicated that dimethoate has a behavior quite different from other insecticides used against *D. oleae*.

In January (*18*) and February (*17*) 1959, Santi and De Pietri-Tonelli announced the existence of a metabolite [*O,O* - dimethyl - *S* - (*N* - methylcarbamoylmethyl) phosphorothioate] (*7*) of dimethoate, a cholinesterase inhibitor stronger than the original compound. Although Dauterman *et al.* (*5*) did not find the same metabolite in rats and lactating cows after oral administration of dimethoate, they suspected its presence because of the occurrence of subsequent cholinesterase depression in vivo. However, further research (*6*) on the persistence of dimethoate and its metabolites following foliar application to plants indicated the presence of the P=O metabolite.

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Research has given a fairly clear picture of the two main metabolic patterns of the active ingredient of dimethoate in plants:

Oxidation, which produces the P=O derivative, a stronger cholinesterase inhibitor and more toxic compound than dimethoate (but less toxic than parathion)

Hydrolytic breakdown, which produces several acid derivatives having negligible biological activity in pests and mammals

Data referring to the acute toxicity of dimethoate to mammals are reported (*8, 9*) and data are given by Santi (*17*) and Dauterman (*5*) for the P=O derivative and the hydrolytic degradation products. Low residues of the P=O metabolite in parts of plants treated with dimethoate and the importance of the toxicological problems connected with the control of *D. oleae* and olive oil production have prompted a deeper investigation of the problem. This article presents the results of investigation on the metabolic fate of P^{32}-dimethoate in olive fruits using chromatography and autoradiography. Findings are interpreted in relation to the safety of olives for oil and eating purposes.

Materials and Methods

Purification of P^{32}-Dimethoate. Because of the critical nature of the research, the purity of samples of P^{32}-dimethoate (Radiochemical Center, Amersham, Bucks, England), was checked by chromatographic and autoradiographic methods. In addition to the main component (about 97%), dimethoate, two more compounds containing P^{32} were found. Therefore, samples were purified before use by automatic countercurrent distribution equipment [water and petroleum ether-benzene (1 + 3) v./v.].

Determination of Metabolism of Dimethoate in Olive Fruits. A sample of P^{32}-dimethoate was formulated as follows: P^{32}-dimethoate, 20 grams; H-23 (wetting agent produced by Montecatini), 1 gram; and methanol, 79 grams. With the formulation diluted with water to dosages recommended for practical use, olive trees were accurately sprayed using a De Vilbiss apparatus. *Frantoio* olive trees were utilized for the investigations on olives yielding oil and *Ascolana tenera* (with relatively small fruits) for eating-olives. Olive trees were sprayed at different times of the year, within the widest limits of the period during which trees are usually sprayed against *D. oleae*.

Samples of olives, picked at various periods of time after spraying, were homogenized and treated with $CHCl_3$ to separate dimethoate, its P=O metabolite, and any other possible derivative which contained P^{32} and which was soluble in that solvent. The extract was designated $CHCl_3$-solubles. The olives were homogenized again and extracted with H_2O to separate the possible radioactive compounds remaining in the tissues after extraction with $CHCl_3$. This extract was designated H_2O-solubles.

The P^{32} content in $CHCl_3$-solubles, H_2O-solubles, $CHCl_3$- and H_2O-insolubles, and the total P^{32} content in the tissues were determined radiometrically and also by the following techniques:

Partition chromatography of the $CHCl_3$-solubles with a silica gel column to separate dimethoate from its P=O derivative by a method described by Dauterman *et al.* (*5*) and Tsuyuki *et al.* (*19*) and adapted for olives

Table I. P.P.M. Dimethoate-P^{32} Equivalents in Olives from Treated Trees

Days between Treatment and Sampling	$CHCl_3$ Solubles			H_2O Solubles	$CHCl_3$ and H_2O Insolubles	Total	γ per 100 Olives[a]
	Total	Dimethoate	P=O derivative				
Olives yielding oil							
P^{32}-dimethoate 0.01% + dimethoate 0.05%, July 8, 1960							
0	16.87	16.87	0.00	...	...	16.87	814.00
4	5.85	5.31	0.54	1.85	0.74	8.44	406.70
12	1.77	...	...	3.11	0.96	5.84	352.39
17	1.40	...	...	3.55	1.63	6.58	549.60
23	1.33	...	...	4.66	2.16	8.15	812.52
45	0.51	...	...	5.92	1.63	8.06	877.12
P^{32}-dimethoate 0.06%, Sept. 15, 1960							
0	11.91	11.91	0.00	...	...	11.91	1741.44
4	4.96	4.20	0.76	1.48	0.30	6.74	962.00
11	3.03	2.00	1.03	3.99	0.66	7.68	1131.83
18	1.70	0.70	1.00	3.55	0.81	6.06	1012.02
25	0.74	...	...	2.88	0.74	4.29	756.80
28	0.59	0.18	0.41	3.15	0.62	4.36	791.36
35	0.74	...	...	3.10	0.74	4.58	811.26
P^{32}-dimethoate 0.06%, Oct. 13, 1960							
0	8.21	8.21	...	...	...	8.21	1826.39
4	8.36	7.72	0.64	0.52	0.14	9.02	2025.38
11	4.14	3.40	0.74	1.33	0.22	5.69	1409.33
20	2.97	1.90	1.07	2.44	0.59	6.00	1446.92
35	1.41	0.73	0.68	2.07	0.59	4.07	1053.91
45	0.59	0.07	0.52	1.92	0.29	2.15	810.60
Olives for eating							
P^{32}-dimethoate 0.06%, Aug. 1, 1960							
0	12.50	12.50	0.00	...	...	12.50	1791.39
2	10.14	10.04	0.10	1.18	0.15	11.47	1615.42
8	4.37	...	...	3.18	0.44	7.99	1342.51
18	1.18	...	...	2.81	0.74	4.73	940.24
26	0.67	0.22	0.45	3.63	1.11	5.41	1724.20
44	0.52	...	...	3.77	1.11	5.40	1381.14
P^{32}-dimethoate 0.06%, Sept. 13, 1960							
0	11.54	11.54	...	...	...	11.54	3096.01
3	7.70	6.35	1.35	1.26	0.67	9.63	2931.36
9	4.66	3.07	1.59	2.59	0.74	7.99	2327.82
21	1.33	0.43	0.90	2.44	1.26	5.03	1628.96
30	0.89	0.27	0.62	3.18	0.81	4.88	1697.93
P^{32}-dimethoate 0.06%, Oct. 17, 1960							
0	8.88	8.88	...	...	...	8.88	2765.31
4	9.62	8.81	0.81	0.96	0.37	10.95	3258.96
11	4.58	3.53	1.05	1.63	0.59	6.80	2210.01
22	2.81	1.73	1.08	1.85	0.74	5.40	1605.58
30	2.81	1.74	1.07	3.40	0.88	7.09	2104.04
44	2.22	0.96	1.26	2.88	0.88	5.98	1884.93
P^{32}-dimethoate 0.04%, Oct. 17, 1960							
0	5.48	5.48	...	...	...	5.48	1609.06
4	6.51	5.85	0.66	...	...	7.18	2056.61
11	2.59	1.75	0.84	1.63	0.22	4.44	1172.53
22	1.26	0.55	0.71	1.55	0.44	3.25	997.15
P^{32}-dimethoate 0.02%, Oct. 17, 1960							
0	2.59	2.59	...	...	...	2.59	744.07
4	2.81	2.48	0.33	...	...	3.40	914.42
11	1.63	1.04	0.59	1.04	0.22	2.89	719.06
22	0.81	0.32	0.49	1.11	0.15	2.07	536.50
30	0.59	0.16	0.43	0.96	0.30	1.85	580.53
44	0.37	...	...	...	...	1.55	430.09

[a] The data indicate variation of content of dimethoate-P^{32} equivalents independent of natural weight increase of olives, in the considered period.

Ascending paper chromatography (using *n*-butyl alcohol saturated with water) with autoradiographic detection of P^{32}-containing compounds to check the components of the radioactive fractions partitioned with silica gel column

Ion exchange separation of the hydrolysis products on Dowex 1-X8, and elution and partition in groups with solvents having an increasing gradient of acidity (*5*, *16*), to separate the compounds containing P^{32} in the H_2O-solubles

Effect of Oil-Yielding Process on Amount and Composition of Residues in Oil. To ascertain the content of dimethoate and of other biologically active compounds in the olive oil, the olives were milled in a small laboratory mill, and the following procedures were used:

Separation of oil from the aqueous phase and olive husks by a laboratory press (Hafico), at a pressure of 450 atm. The oil extracted approximated 70 to 80%

Clarification of the oil in a laboratory centrifuge at 4000 r.p.m. (about 1700 × G)

Determination of the radioactivity and P^{32} concentration (p.p.m.) in the oil, aqueous phase, and olive husks

Extraction from the oil (with a hydro-alcoholic solution containing 50% ethyl alcohol) (*4*) of dimethoate and its P=O metabolite possibly present; paper chromatography of the extracts and autoradiographic detection of the above-mentioned compounds in the oil

Extraction, in the aqueous phase and olive husks, with $CHCl_3$ of dimethoate and its P=O metabolite possibly present; quantitative partition with a silica gel column (*5*, *19*) and radiodetermination of the amount of the above-mentioned compounds in the aqueous phase and olive husks

Influence of Industrial Processing of Eating-Olives with NaOH on Residue Levels. Olives, previously sprayed in the field with dimethoate, were soaked in a 1.8% NaOH solution for 24 hours; the alkaline solution was removed, and the olives were soaked in water for 5 days. The water was changed every 4 hours. The olives were then transferred into a 10% NaCl solution for 15 days (*12*). The concentration of P^{32} in the olives was radiodetermined just before the NaOH treatment, periodically after soaking in NaOH solution, at the end of the NaOH treatment, and when the fruits were transferred into the brine.

Results and Discussion

The results were obtained under conditions much more drastic than when dimethoate is practically applied against *D. oleae*. The most unfavorable conditions are:

P^{32}-dimethoate, diluted in water, was applied with a De Vilbiss spraying apparatus to deposit a maximum amount of insecticide on fruits, foliage, and twigs. In practical conditions, with normal spraying equipment and adopting a very accurate technique, it is almost impossible to reach the initial deposit achieved here.

In research on the metabolism of dimethoate in eating-olives and on the influence of NaOH treatment, *Ascolana tenera*, a variety of olives having small fruits (350 per kg. when they are ripe), was chosen. With the same dosage of active ingredient in the liquid to be applied, the highest initial deposit on the

Table II. Ion Exchange Separation on Dowex 1-X8 of P^{32} from H_2O Solubles in Olives for Eating

Eluents	Days between Treatment and Sampling[a] 21 (Treatment Sept. 13, 1960)	30 (Treatment Sept. 13, 1960)	11 (Treatment Oct. 17, 1960)	22 (Treatment Oct. 17, 1960)	Probable Compounds Which, if Present, Can Be Eluted
	% P^{32}				
0.01*N* HCl 0.1*N* HCl	82.71	68.18	90.92	73.11	H_3PO_4; $(CH_3O)_2P(=O)OH$; $(CH_3O)_2P(=O)OH$
0.1*N* HCl + CH_3OH, 1:3 1*N* HCl + CH_3OH, 1:3	13.44	23.05	7.44	17.94	$(CH_3O)_2P(=S)OH$; $(CH_3O)_2P(=S)SCH_2COOH$
1*N* HCl + CH_3COCH_3, 1:3 Concd. HCl + H_2O + CH_3COCH_3, 1:1:6	3.59	7.34	1.41	8.24	$(CH_3O)(HO)P(=S)SCH_2CON(H)CH_3$; $(CH_3O)_2P(=S)SH$
Concd. HCl + H_2O + CH_3COCH_3, 1:1:6	0.00	1.02	0.00	0.04	$(CH_3O)_2P(=S)SH$
Radioactive residues in column	0.26	0.41	0.23	0.67	

[a] Percentage of P^{32} attached to Dowex 1-X8: 90.29, 91.50, 91.45, and 90.40%.

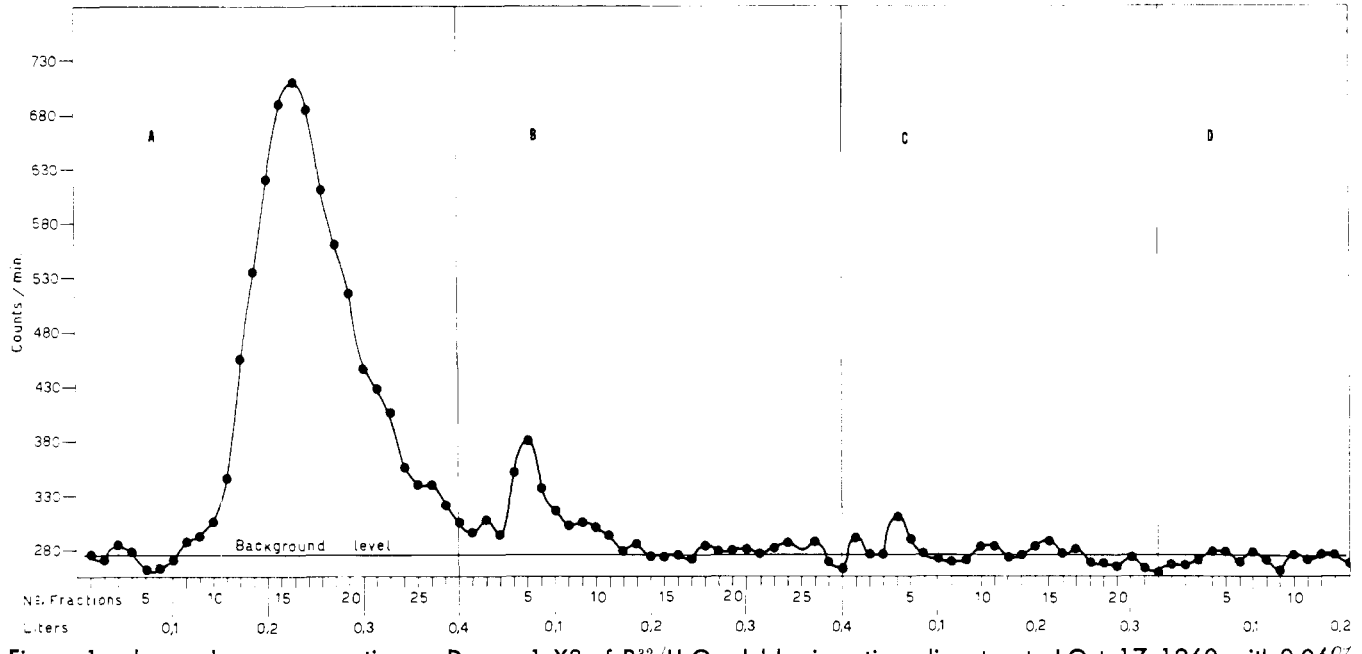


Figure 1. Ion exchonge separation on Dowex 1-X8 of P^{32}/H_2O solubles in eating-olives treated Oct. 17, 1960, with 0.06% P^{32}-dimethoate and sampled 11 days after treatment

A. From 0.01*N* HCl to 0.1*N* HCl
B. From 0.1*N* HCl–CH_3OH (1:3) to 1*N* HCl–CH_3OH (1:3)
C. From 1*N* HCl–CH_3COCH_3 (1:3) to concd. HCl–H_2O–CH_3COCH_3 (1:1:6)
D. From concd. HCl–H_2O–CH_3COCH_3 (1:1:6)

fruits was attained as the smallest fruits have a maximum surface-to-weight ratio.

Oil was extracted from the sprayed fruit samples after milling, but without adding any water (at a convenient temperature), as is done commercially. In fact, the addition of water causes the residues of dimethoate to pass from the oil to the aqueous phase.

The highest dosage of active ingredient (0.06%) was used which is recommended only for the protection of olives yielding oil and is applied at the end of September, to maintain an effective control until the attack of the fly is over and the fruits are picked. In all other cases, lower dosages are recommended.

The influence of the industrial oil-yielding process on the levels and nature of residues in the oil was investigated with olives treated in the middle of October—i.e., at the latest recommended time. At that time of the year, the degradation of dimethoate is reduced to a minimum, and a very low dosage (0.02% of active ingredient) is sufficient until the fruits are picked.

The residues ascertained in these experiments must, therefore, be considered as the highest values which can be reached only under exceptional field and environmental conditions.

The first applications were made in July for the olives yielding oil (Table I), and in August for the eating olives (Table II). Owing to the low specific radioactivity of the P^{32}-dimethoate used, the concentrations of dimethoate and its P=O metabolite could be determined only at short periods of time after treatment. Nevertheless, the total data indicate the metabolic changes of the

insecticide in the olive and the variation of content of the principal degradation substances as a function of the dosage and timing of application.

For consumption of the fruits picked from the trees, which however is quite unusual, the residue levels of dimethoate and of its P=O metabolite in the olives are of no toxicological concern if the trees are sprayed at a dosage not higher than 0.06% from the beginning to the middle of September and at a dosage of 0.02% at the end of that period (middle of October) and if the olives are utilized 1 month or more after treatment. When lower dosages are used, the interval between the last treatment and harvest can be reduced.

The active ingredient penetrates and diffuses rapidly into the fruits and undergoes oxidation and hydrolysis. Its concentration decreases at a rate which, starting from the application of the insecticide, has a maximum and a minimum at the beginning and end of the considered period of time, respectively.

The P=O metabolite is detectable shortly after treatment; its concentration increases to a maximum and then gradually decreases. On the contrary, the concentrations of the products of hydrolysis (acid and hydrosoluble compounds) increase, while those of dimethoate and its P=O metabolite decrease (Table I). Although analytical research has not yet been completed, it can be stated that, according to behavior on ion exchange separation columns with eluents having an increasing gradient of acidity (Table II and Figure 1) and by paper cochromatography (Table III), the dimethyl phosphoric acid is absent. However, the small difference between the R_f values gives no evidence of the presence of phosphoric acid or its monomethyl derivative or both.

At present, there is no experimental evidence on the nature of the small amount of the P-containing substance(s) derived from dimethoate and not extracted with $CHCl_3$ and with H_2O. As has been demonstrated in other similar cases, the plants may have synthetized organic compounds with those P-containing substances.

Research to determine the influence of the process of oil yielding on residues in oil has clearly demonstrated that oil is free from the P=O metabolite and from any other P^{32}-containing metabolite (Table IV), but in oil obtained from olives treated (September 15, 1960) with P^{32}-dimethoate 0.06% the concentrations of dimethoate, 11 and 44 days after treatment, are 0.46 and 0.08 p.p.m., respectively.

Residue levels of dimethoate in oil depend on the concentration of active ingredient in the olives, indirectly on the dosage of dimethoate applied on the trees, and on the time elapsed between treatment and process of oil-yielding olives.

In general, the concentration of dimethoate in the oil represents about $^1/_3$ to $^1/_4$ of the concentration of active ingredient in the olives. Since, following the schedule recommended for using the insecticide against *D. oleae*, the concentration of dimethoate in the olives

Table III. R_f Values of P^{32}/H_2O Solubles Exchanged with the First Elution Gradient on Dowex 1-X8 and R_f Values of Probable Compounds for Comparison P^{32}/H_2O Solubles Obtained from Olives for Eating Sprayed with P^{32}-Dimethoate, Oct. 17, 1960, and Picked 11 Days after Treatment

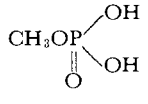
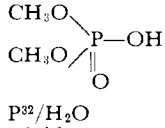
Compounds	2-Propanol–Water–Ammonia, Sp. Gr. 0.909 (74:25:1, by Vol.)	Acetonitrile-Water (80:20, by Vol.)
H_3PO_4	0.06	0.04
	0.09	0.06
	0.47	0.15
P^{32}/H_2O solubles	0.00	0.02

Table IV. P.P.M. Dimethoate-P^{32} Equivalents as Dimethoate and Its P=O Derivative in the Oil, Aqueous Phase, and Olive Husks Obtained from Olives Treated Oct. 13, 1960, with P^{32}-Dimethoate 0.06%

Days between Treatment and Sampling	Oil[a]	Aqueous Phase			Olive Husks		
		$CHCl_3$-solubles			$CHCl_3$-solubles		
	Dimethoate	Dimethoate	P=O derivative	$CHCl_3$-insolubles	Dimethoate	P=O derivative	$CHCl_3$-insolubles
7	1.80	4.15	0.66	2.29	5.88	0.32	1.26
14	0.90	2.26	1.21	4.59	3.36	0.49	1.18
25	0.40	0.56	1.23	7.18	1.05	0.33	1.70
35	0.22	0.29	0.91	6.96	0.75	0.29	2.59
45	0.10	0.14	0.62	5.70	...	...	1.85

[a] All results with P=O derivative were zero.

Table VI. P.P.M. Dimethoate-P^{32} Equivalents in Olives Soaked in Water (5 Days) after the Treatment with 1.8% NaOH

% Concn. of P^{32}-Dimethoate Sprayed on Trees	P.P.M. Dimethoate-P^{32} in Olives				% P^{32} in olives soaked for 24 hours in 1.8% NaOH and in H_2O for 5 days (referred to total P^{32} content in the olives before dipping into the NaOH solution) (24 hours after picking)
	Before dipping into 1.8% NaOH (24 hours after picking)	After 24 hours in 1.8% NaOH	In 1.8% NaOH and in H_2O for 5 Days		
			$CHCl_3$-solubles	$CHCl_3$-insolubles	
0.06	6.91	1.26	0.02	0.13	2.16
0.04	5.01	0.85	0.00	0.10	1.99
0.02	1.74	0.18	0.00	0.017	0.98

Table V. Dimethoate-P^{32} Equivalents in Eating-Olives during and after 1.8% NaOH Treatment

Olives sprayed Oct. 17, 1960, and picked 30 days after treatment

Concentration of P^{32}-Dimethoate Sprayed on Trees, %	P.P.M. Dimethoate-P^{32} Equivalents in Olives at Harvest				P.P.M. Dimethoate-P^{32} Equivalents in Olives Treated with 1.8% NaOH after:			% P^{32} Extracted from 1.8% NaOH Solution after:		
	Dimethoate	P=O derivative	H_2O solubles	$CHCl_3$ and H_2O insolubles	3 hours	7 hours	24 hours	3 hours	7 hours	24 hours
0.06	1.74	0.88	3.40	0.89	6.91	5.70	1.26	0.00	17.51	81.76
0.04	1.00	0.75	2.62	0.74	5.01	3.95	0.85	0.00	21.15	83.03
0.02	0.16	0.32	0.96	0.30	1.74	1.35	0.18	0.00	22.41	89.65

[a] Referred to total P^{32}-content on olives before dipping into NaOH solution.

when the fruits are picked reaches a maximum ranging from 0.1 to 0.2 p.p.m., the many analyses of oil samples from several olive-growing areas have constantly shown the practical absence of residues of dimethoate and its metabolites.

Research has given clear evidence that a certain amount of dimethoate—almost the whole amount of the P=O metabolite and of the products of hydrolysis—is contained in the aqueous phase. The olive husks contained a part of the dimethoate (whose concentration gradually decreases with increasing elapsed time between treatment and process of oil yielding) and traces of the P=O metabolite and of compounds extracted with $CHCl_3$ (Table IV).

Treatment of eating olives with NaOH, in the same way as in the industrial process, produces further degradation and a strong extraction of the P^{32}-containing compounds possibly present in the fruits.

At the end of the soaking in NaOH solution, the amount of P^{32} extracted represents about 85 to 90% of the content (referring to the residues of dimethoate and its metabolites) present in the olives at the moment of dipping (Table V). A further soaking of olives in water for 5 days (as usually recommended in the industrial process) takes out from 98 to 99% P^{32} derived from residues of dimethoate in olives at harvest time (Table VI).

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QUALITY OF SHUCKED OYSTERS

Chromate Color Test for Estimating Age-Temperature History of Raw Shucked Oysters

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COMMERCIALLY shucked raw oysters are among the most perishable food products. Since the sanitary safeguards normally used with food products generally cannot be used with raw oysters, only good sanitary practices during harvesting, shucking, storing, and transportation can assure the consumer safe and palatable oysters. Control measures have been developed as a result of disease outbreaks in 1924–25, 1928, and 1939, attributable to bacterial contamination from oysters. The shellfish industry, local health authorities, and the Public Health Service have cooperated in setting tentative standards for permissible levels of viable bacteria and certain coliform organisms in shellfish growing waters, in the oysters before shucking, and in the finished product. Satisfactory compliance by interstate shippers is recognized by state certification.

Although no satisfactory chemical tests are available to indicate the sanitary quality of oysters, pH is used frequently as an indicator of acceptability and potential storage life. The pH of freshly shucked oysters appears, however, to be dependent in part on harvesting area and season. Freshly shucked oysters from Apalachicola Bay in the Gulf of Mexico may vary from pH 6.0 in late spring to 6.4 in winter (*5*); the pH of oysters from the East Coast may be as high as 6.8 and is apparently independent of seasonal variation (*7*). The pH of oysters from Chesapeake Bay usually decreases during optimum storage, whereas that of oysters from the Gulf Coast may not change for several days (*1, 5*).

The storage life of raw shucked oysters depends primarily on storage temperature. The manual of recommended practices (*11*) suggests that shucked oysters be cooled to 10° C. or lower within 2 hours after shucking and held at that temperature during storage. The freshly packaged oysters usually are cooled with crushed ice; dry refrigeration also is used. The time required to cool packaged oysters is dependent on the temperature and capacity of the refrigeration system, the initial temperature of the oysters (which is largely dependent on the temperature of the water used for washing and "blowing"), and the size of the container. In a crushed ice slurry over 3 hours is required to cool a 1-gallon can of oysters from 17° to 10° C., whereas less than 30 minutes is required to cool a $^1/_2$-pint can (*10*). Rigorous control of storage is very difficult because of the time and distance between the harvesting and sale of oysters. Although 3 or 4 weeks may expire before oysters at 0° C. show signs of decomposition, definite organoleptic signs of spoilage appear much earlier if oysters are allowed to reach temperatures above 10° C. during storage.

During work on other projects related to shellfish, substances capable of acting as reducing agents in a variety of oxidation-reduction reactions were observed in the liquor surrounding commercially shucked oysters. Preliminary investigations indicated that these substances included glucose, lactic acid, and