Agricultural Experiment Station: Lexington, KY, 1940.
Lowe, R. H.; Gillespie, M. An *Escherichia coli* Strain for use in Nitrate Analyses. *J. Agric. Food Chem.* **1975**, *23*, 783-787.
MacKown, C. T.; Douglass, B.; Djordjevic, M. V.; Bush, L. P. Tobacco-Specific N-Nitrosamines: Formation during Processing of Midvein and Lamina Fines. *J. Agric. Food Chem.* **1988**, *36*, 1031-1035.
Mirvish, S. S. Formation of N-nitroso Compounds: Chemistry, Kinetics and *in vivo* Occurrences. *Tox. Appl. Pharm.* **1975**, *31*, 321-251.
Parsons, L. L.; Smith, M. S.; Hamilton, J. L.; MacKown, C. T. Nitrate Reduction during Curing and Processing of Burley Tobacco. *Tob. Sci.* **1986**, *30*, 100-103.
Tso, T. C. *Physiology and Biochemistry of Tobacco Plants*; Dowden, Hitchinson, and Ross: Stroudsburg, PA, 1972.
Walton, L. R.; Casada, M. E.; Swetnam, L. D.; Casada, J. H. Diffusion of Moisture from Curing Tobacco Leaves. *Tran. Am. Soc. Agric. Eng.* **1982**, *25*, 1099-1102.

Received for review June 17, 1988. Accepted September 8, 1988.

Dissipation of Isomalathion on Solid Pesticide Carriers, Container Surfaces, and Leaves and Some Degradation Products of Isomalathion on Carriers[1]

S. Rengasamy* and Balraj S. Parmar

Isomalathion undergoes degradation on solid pesticide formulation carriers, container surfaces, and *Vigna radiata* L. leaves. Nearly 40–50% of the applied isomalathion was lost on carriers bentonite, kaolinite, hydrated calcium silicate, and silica gel H on 12-day incubation at 55 ± 1 °C. The loss was 8–18% on different container surfaces under the same conditions. On *V. radiata* L. leaves, nearly 99% of the applied isomalathion was lost within 5 days of application, the loss being initiated within 30 min of application. Of the several degradation products formed on carriers, *O,S,S*-trimethyl phosphorodithioate, diethyl mercaptosuccinate, and diethyl methylmercaptosuccinate were identified.

Since the 1976 malathion poisoning episode in Pakistan (Baker et al., 1978), several studies on the formation of isomalathion in malathion products have been reported (Miles et al., 1979, 1980; Verschoyle et al., 1982; Halder, 1982; Halder and Parmar, 1984; Rengasamy and Parmar, 1988). Some of these studies indicated the instability of isomalathion (Halder, 1982; Halder and Parmar, 1984). However, no systematic information on its fate has been reported. In the present study, the loss of isomalathion on different carriers, container surfaces, and *Vigna radiata* L. leaves has been reported. An effort has been made to identify some of the transformation products of isomalathion on solid carriers.

MATERIALS AND METHODS

Isomalathion was prepared from technical malathion as per details reported earlier (Rengasamy and Parmar, 1988). Bentonite, kaolinite, hydrated calcium silicate, and silica gel H used in this study were also detailed earlier (Rengasamy and Parmar, 1988).

Isomalathion Loss on Carriers. A 10-mg portion of isomalathion in chloroform was applied to 1 g of each carrier, worked into a paste, dried for 24 h under ambient conditions (31 °C, RH 52%), and ground to a fine powder and 100 mg of powder (0 day sample) withdrawn from each. The powders were then incubated in bakelite screw-capped sample vials at 55 ± 1 °C for 12 days and sampled periodically. The samples were extracted in 5 mL of chloroform and filtered (Whatman No. 42), a suitable aliquot of the filtrate was withdrawn, and solvent was removed on a rotary evaporator. The residue was dissolved in 2 mL of 2-propanol and analyzed for isomalathion content. The percent loss of isomalathion was calculated on the basis of quantity applied and recovered.

Isomalathion Loss on Container Surface. A borosilicate glass Petri dish (diameter 5 cm, height 1 cm), aluminum box (diameter 5 cm, height 1 cm), and high-density polyethylene bottles (diameter 5 cm, height 5 cm) provided the test surfaces. Chloroform solution containing 500 μg of isomalathion was spread in each of the seven containers per test surface. Chloroform was evaporated under a ceiling fan to obtain a thin film. One sample per test surface was worked up immediately (0 day), and the rest of the containers were incubated at 55 ± 1 °C for 12 days. One container under each test surface was periodically withdrawn, the contents were dissolved in 2 mL of 2-propanol, and isomalathion content was analyzed. The percent loss of isomalathion was calculated as above.

Isomalathion Loss on Leaf Surface. Seven 40-day-old green gram (*V. radiata* L.) plants were selected in field, and three trifoliated leaves per plant were treated with 1000 μg of isomalathion in 2:1 methanol–water, using a pipet. One plant (three trifoliated leaves) was plucked per sampling at 30 min (0 day) and at 1, 2, 3, 4, 5, and 6 days after treatment. The treated leaves were macerated with 5 g of anhydrous Na_2SO_4 and extracted in 75 mL of chloroform. The extract was concentrated to 10 mL on a rotary evaporator and passed through a column of charcoal–anhydrous Na_2SO_4 (5 + 5 g) admixture sandwiched between anhydrous Na_2SO_4 layers. The column was eluted with 50 mL of chloroform, the solvent removed in a rotary evaporator, residue dissolved in 2 mL of 2-propanol, and isomalathion content analyzed. The recovery of isomalathion when applied to cleanup column was 99%. It was 35% from leaves after 30 min of application.

A Kontron Analytic liquid chromatograph, Series 640, from Kontron Electrolab, London, England, equipped with

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India.
[1] Contribution No. 409.

0021-8561/89/1437-0430$01.50/0

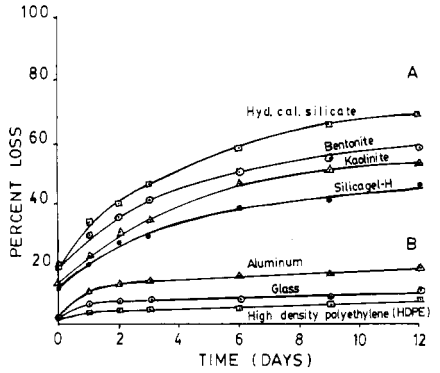


Figure 1. Loss of isomalathion on different carriers (A) and containers (B).

a Uvikon 730 S LC variable-wavelength UV detector and millivolt recorder was used. Operating conditions: eluant, isooctane–2-propanol–dichloromethane (9:1:0.05); flow rate, 1.2 mL min^{-1}; column material, Lichrosorb Si-60, 10 μm, steel column (250 × 4.9 mm); injection volume, 20 μL; detector, 222 nm; attenuation, 0.08 AUFS; recorder sensitivity, 10 mV fsd; chart speed, 10 mm min^{-1}. Retention time for isomalathion was 10.5 min.

Isomalathion Degradation Products on Carriers. Isomalathion (100 mg) in chloroform was impregnated on 5 g each of bentonite, kaolinite, hydrated calcium silicate, and silica gel H, dried in a current of air at 25 °C, and ground to a fine powder. After 36 h of impregnation, the powders were incubated in bakelite screw-capped sample vials at 55 ± 1 °C for 48 h and extracted in 25 mL of chloroform. The extract was evaporated in a rotary evaporator at 50 °C, residue dissolved in chloroform, and the volume made up to 2 mL. Aliquots were analyzed by gas chromatography–mass spectrometry (GC–MS).

Gas Chromatography–Mass Spectrometry. A JEOL Inc. Model JGC-20 K gas chromatograph interfaced with JMS-D 300 mass spectrometer (equipped with a computer, Model JMA 300) was used. The gas chromatograph (GC) fitted with a coiled glass column (3 mm (i.d.) × 1 m) packed with 5% SE 30 on Gas Chrom Q was set at gas (He) flow back pressure, 1 kg cm^{-2}; injection port and interphase temperatures were 220 °C. Oven temperature was programmed from 70 °C for 5 min to 200 °C at 5 °C min^{-1}. The mass spectrometer (MS) was set for electron impact (EI) spectrum at ionization chamber temperature, 210 °C. Operating conditions: ionization current, 100 μA; ionization beam, 70 eV; accelerating voltage, 3 kV; scan range, m/z 50–500; scan speed, 5 s.

Mass spectra of malathion and isomalathion were taken by the direct insertion method. Aliquots (1–2 μL) of isomalathion (1000 ppm) solution in chloroform and the extracts of clay-treated samples above were injected into the GC of the GC–MS. The column effluent was diverted at the interface for the first 90 s. The computer on line with the ion collector of the MS accumulated the data.

RESULTS AND DISCUSSION

Loss of Isomalathion. Isomalathion loss on different carriers is shown in Figure 1. Nearly 45–50% of the applied isomalathion was lost during the 12-day incubation. Maximum loss occurred on hydrated calcium silicate followed by bentonite, kaolinite, and silica gel H. The mode of loss appears primarily to be chemical degradation as seen later in this paper. In earlier studies by Halder and Parmar (1984), Rengasamy (1986), and Rengasamy and Parmar (1988) minimum isomalathion content was observed in malathion-hydrated calcium silicate powder. The present results point out that isomalathion does not remain stable on this carrier. Its use for formulating malathion powders may be preferred from the point of view of isomalathion content.

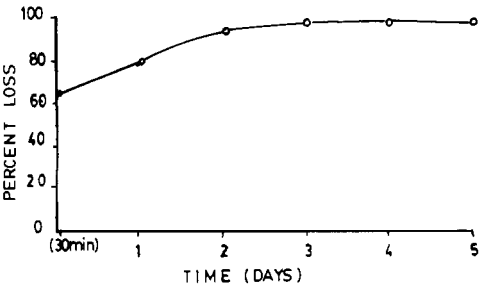


Figure 2. Isomalathion loss on green gram, *V. radiata* L., under field conditions.

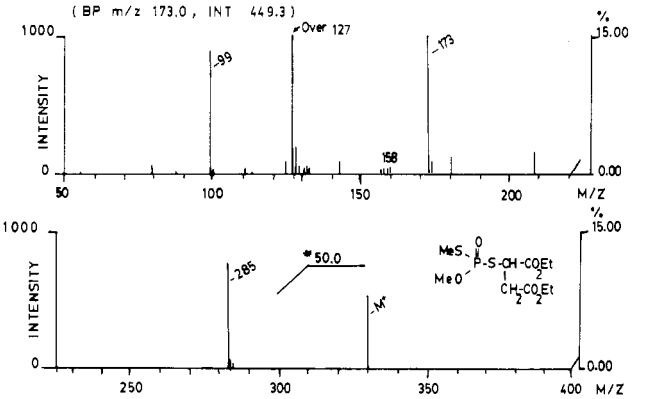


Figure 3. Mass spectrum of isomalathion.

Isomalathion loss on container surfaces was only 8–18% during the 12-day incubation at 55 ± 1 °C (Figure 1). The loss was maximum on aluminum surface followed by borosilicate glass and high-density polyethylene (HDPE). In an earlier study (Rengasamy and Parmar, 1988), malathion films applied on borosilicate glass showed maximum isomalathion content (1.09%) as compared to aluminum (0.48%) and HDPE (0.19%). This points to the likelihood of isomalathion accumulation in malathion stored in glass containers. However, malathion stored in borosilicate glass vials at 55 ± 1 °C for 12 days showed a maximum of only 0.42% isomalathion content (Rengasamy, 1986; Rengasamy and Parmar, 1988), indicating the catalytic effect of glass container to be limited to the glass–malathion interface only.

Under field conditions, isomalathion was lost at a very fast rate on green gram leaves. Of the 1000 μg applied only about 345 μg was recoverable after 30 min. Nearly 99% was lost by the 5th day (Figure 2), suggesting the remote possibility of isomalathion accumulation on malathion-treated leaves. In another study, when malathion was applied to leaves at 500 times the normal application dose, the isomalathion content was below the detectable level of 0.2 ppm (Rengasamy and Parmar, 1988). This indirectly supports the isomalathion instability under field conditions.

Degradation Products on Carriers. Malathion showed mass spectral peaks at m/z 330 (M^+), 285 (M^+ – 45), 173 (100%), 158, 127, and 93 that compared well with those reported by Damico (1966). Mass spectral fragments of isomalathion showed MS peaks at m/z 330 (M^+), 285 (M^+ – 45), 173, 158, 127 (100%), and 99 (Figure 3, and Scheme I).

The mass chromatograms of isomalathion (neat and after treatment with silica gel H) are reported in Figure 4. Peaks a, b, f, and n were found in the reference as well as in the treated samples. Peak n (reconstructed) corre-

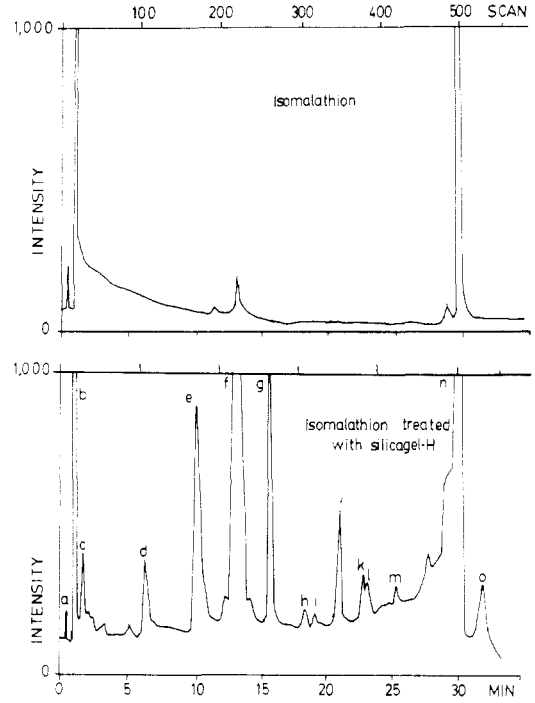


Figure 4. Mass chromatograms of isomalathion.

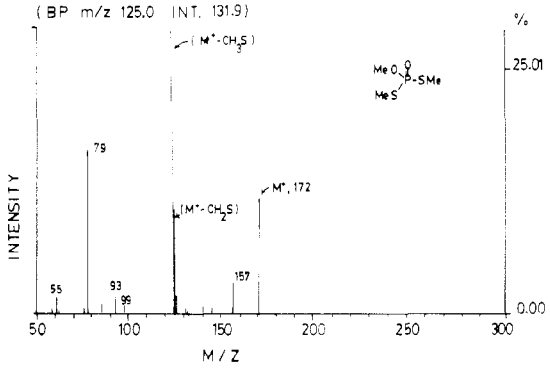


Figure 5. Mass spectrum of compound e.

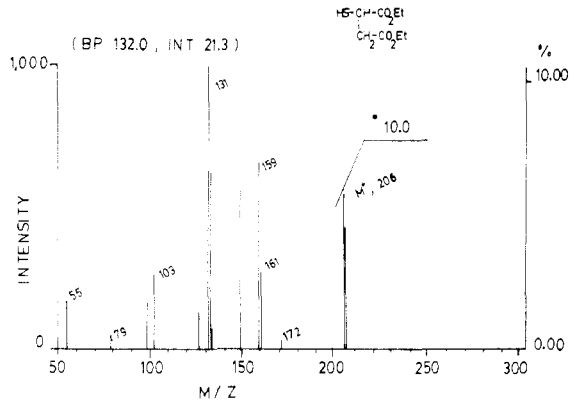


Figure 6. Mass spectrum of compound f.

sponds to isomalathion. Peak e appeared as a degradation product of isomalathion. Its mass spectra (Figure 5) showed peaks at m/z 172 (M^+), 157 ($M^+ - CH_3$), 126 ($M^+ - CH_2S$), 125 ($M^+ - SCH_3$) (100%), 99, 93, and 79. It was identified as *O,S,S*-trimethyl phosphorodithioate (OSS, VII). Compound f showed mass spectral peaks (Figure 6) at m/z 206 (M^+), 172 ($M^+ - H_2S$), 161, 159, 131 (100%), 103, 79, and 55. It was identified as diethyl mercaptosuccinate (II). The concentration of this product increased on treating isomalathion with silica gel H. Compound g, also formed on treatment with the carrier, showed mass

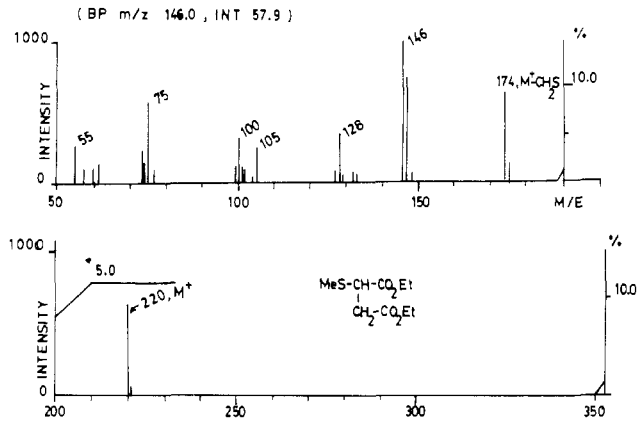
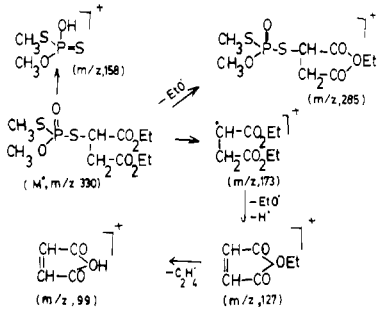
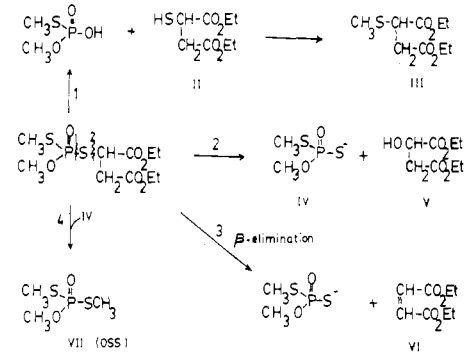


Figure 7. Mass spectrum of compound g.

Scheme I. Proposed Mass Spectral Cleavage of Isomalathion



Scheme II. Proposed Degradation Route of Isomalathion on Carriers



spectral peaks (Figure 7) at m/z 220 (M^+), 174, 146 (100%), 128, 105, 100, 75, and 55. It was identified as diethyl methylmercaptosuccinate (III). Compounds corresponding to peaks a–d,i–m,o could not be identified due to lack of reference mass spectral data.

Isomalathion on treatment with bentonite, kaolinite, and hydrated calcium silicate showed the same degradation pattern with apparently different rates as indicated by their mass chromatograms.

Based on the above data, a degradation pathway of isomalathion on clays is proposed in Scheme II. Hydrolytic route 1 forms products f (II) and dimethyl phosphorothioate (I). Methylation of f probably by isomalathion leads to g (III). Hydrolysis by route 2 forms diethyl malate (V) and ion IV. β-Elimination (route 3) produces diethyl fumrate (VI).

Of the possible degradation products of isomalathion, *O,O,S*-trimethyl phosphorothioate and OSS are reported to be toxic to mammals, the acute oral LD_{50} to rats being 60 and 26 mg/kg, respectively, as compared to malathion (pure) (12 500 mg/kg) and isomalathion (113 mg/kg). This

study confirms the formation of OSS from isomalathion in concentrations depending on the carrier used. It can probably modify the potentiating activity of isomalathion also (Verschoyle et al., 1982). OSS being a stable product, monitoring its contents in malathion powders may be desirable.

ACKNOWLEDGMENT

S.R. thanks the Indian Council of Agricultural Research for the award of Senior Research Fellowship during the course of these studies.

Registry No. II, 23060-14-2; III, 1642-46-2; VII, 22608-53-3; isomalathion, 3344-12-5; kaolinite, 1318-74-7; calcium silicate, 1344-95-2; polyethylene, 9002-88-4.

LITERATURE CITED

Baker, E. L.; Zack, M.; Miles, J. W.; Alderman, L.; Warren, Mc. W.; Dobbin, R. D.; Miller, S.; Teeters, W. R. Epidemic Malathion Poisoning in Pakistan Malaria Workers. *Lancet* **1978**, 31–34.

Damico, J. N. The mass spectra of some organophosphorus pesticide compounds. *J. Assoc. Off. Anal. Chem.* **1966**, *57*, 165–167.

Halder, A. K. Effect of Carrier on Isomalathion Formation. Ph.D. Dissertation, Post Graduate School, Indian Agricultural Research Institute, New Delhi, India, 1982.

Halder, A. K.; Parmar, B. S. Effect of Carrier on Isomalathion Formation in Malathion Powders. *J. Pestic. Sci.* **1984**, *9*, 147–150.

Miles, J. W.; Mount, D. L.; Staiger, M. A.; Teeters, W. R. *S*-Methyl Isomer Content of Stored Malathion and Fenitrothion Water-Dispersible Powders and Its Relationship to Toxicity. *J. Agric. Food Chem.* **1979**, *27*, 421–425.

Miles, J. W.; Mount, D. L.; Churchill, F. C. The Effect of Storage on the Formation of Minor Components in Malathion Powders. Presented at the 24th Annual Meeting of the Collaborative International Pesticide Analytical Council, Granada, Spain, 1980.

Rengasamy, S. Formation and Transformation of S-Methyl Isomer of Malathion in Technical Malathion and Malathion Products. Ph.D. Dissertation, Post Graduate School, Indian Agricultural Research Institute, New Delhi, India, 1986.

Rengasamy, S.; Parmar, B. S. Investigation of Some Factors Influencing Isomalathion Formation in Malathion Products. *J. Agric. Food Chem.* **1988**, *36*, 1025–1030.

Verschoyle, R. D.; Reiner, E.; Bailey, E.; Aldridge, W. N. Dimethylphosphorothioates: Reaction with Malathion and Effect on Malathion Toxicity. *Arch Toxicol.* **1982**, *49*, 293–301.

Received for review April 7, 1988. Accepted September 15, 1988.

New Approach To Improve the Gelling and Surface Functional Properties of Dried Egg White by Heating in Dry State

Akio Kato,* Hisham Radwan Ibrahim, Hiroyuki Watanabe, Kazuo Honma, and Kunihiko Kobayashi

The controlled heating in dry state was very effective to improve the gelling and surface functional properties of dried egg white. After heating at 80 °C in dry state (7.5% moisture content) for 7 days, the solubility of dried egg white was not affected and the Maillard reaction was not detectable. The surface hydrophobicity increased with heating time. The foaming and emulsifying properties increased in proportion to increases in heating time, correlating with surface hydrophobicity. The gel strength increased about 4 times with heating for 10 days. The electrophoretic patterns of heat-treated dried egg white revealed the formation of hydrophobic and disulfide protein–protein interaction. A mild change was observed in the CD spectrum when dried egg white was heated in dry state. Thus, the heating in dry state seems to be a new approach to improve the functional properties of food proteins.

Egg white proteins are extensively utilized as functional food products in food processing. For application in food processing, the pasteurization of liquid egg white is usually carried out for a few minutes at temperature near 60 °C. However, because of high heat sensitivity of egg white proteins, the functional property such as whipping property begins to be damaged at temperatures as low as 54 °C (Cunningham and Lineweaver, 1965). Dried egg white also generally receives heat treatment at 55–65 °C to reduce microbial numbers. The effect of heating on egg white at pasteurization temperature has been studied by many researchers (Seideman et al., 1963; Garibaldi et al., 1968; Cunningham and Lineweaver, 1965, 1967; Chang et al., 1970; Cunningham, 1974). However, they studied the effect of heating on egg white only in solution. There are no studies about the effect of heating in dry state on the functional properties of egg white proteins. It is of interest to know the effect of heating in dry state on the functional properties of dried egg white by storing at various temperature in dry state for different times. Thus, we found a significant improvement of functional properties such as the foaming, emulsifying, and gelling properties of dried egg white by heating in dry state at 80 °C for several days. This finding may make possible to expand further applications of dried egg white in food processing. In addition, this phenomenon is interesting in terms of the investigation of conformational changes of proteins with heating in dry state.

Department of Agricultural Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan (A.K., H.R.I., K.K.), and Q. P. Corporation, Fuchu-shi, Tokyo 183, Japan (H.W., K.H.).

MATERIALS AND METHODS

Egg white (DEW) spray-dried at 60–70 °C after decarbohydrate treatment was provided by Q. P. Corp., Tokyo. Heat treatment of DEW was done as follow: 5 g of DEW was put in a test tube tightly sealed and then incubated at 80 °C for various periods of time (day) in dry state (7.5% moisture content). As soon as the sample was

0021-8561/89/1437-0433$01.50/0