Recent Problems on Food Contamination in Japan

Takashi Suzuki

National Institute of Hygienic Sciences, Japan

I. ENVIRONMENTAL POLLUTION BY ORGANOTIN COMPOUNDS

INTRODUCTION

Outline—Organotin compound is one of the most widely used organometalic compounds, and its annual production has been increasing year by year since the use as the stabilizer for PVC was found in USA in 1945. Its use has increased rapidly especially since the biocidal activity of trialkyltin compounds (R_3Sn^+) was found in the 1950's[1]. As R_3Sn^+ have been used for fish net and ship paint for its property, the organotin compound is giving a serious effect on marine products in the sea near the shore[2-4].

Chemical properties of organotin compounds—When organotin compounds are classified according to their chemical structures, they are expressed as $RsnX_3$, R_2SnX_2, R_3SnX, and R_4SnX, in which X expresses halogens, short or long chain fatty acids, esters in the case of dicarboxylic acid, mercaptans, and mercaptocarboxylic acids, and R expresses alkyl or aryl chains. The Sn-C bond is relatively stable compared with the Sn-X bond. On the other hand, the Sn-X bond has higher ionic character compared with the Sn-C bond, and therefore X is easily replaced by other anions. The Sn-X bond have also a tendency to form adducts, which are unstable and easily decomposed by strong acids or strong bases, with anmmonia or amine. Organohalogenotin compounds also form oxides when they are treated with NaOH. For example, monbutyltin chloride ($BuSnCl_3$), dibutyltin dichloride (Bu_2SuCl_2), and tributyltin chloride (Bu_3SnCl) form butylmetastannoic acid ($BuSnOOH$), dibutyltin oxide (Bu_2SnO), and bis (tributyl) tin oxide ($(Bu_3Sn)_2O$, TBTO), respectively. These compounds are reversibly converted to original compounds by HCl. Among them methyl (Me), butyl (Bu), octyl (Oct), and phenyl (Ph) compounds are industrially important.

Uses—The industrial use of $RSnX_3$ are quite few. On the other hand R_2 SnX_2 type compounds are important not only as intermediates of synthesis of organotin compounds, but also as the stabilizers for PVC to protect from heat and light. It has an application as catalyst for polymers such as polyesters and silicon elastomers. The compounds, R_3SnX, have an important application as biocides. They are used as antifoulants of fish net and ship paints, wood preservatives, and slimicides. Generally speaking, the kind of X does not have much effect on the biological activity of organotin compounds, but the effect on the biological activity of organotin compound is the strongest when the sum of the number of carbon is 9 to 12 when R is a alkyl moeity[5]. This means the activity is the highest when alkyl moeity is propyl or butyl in R_3SnX. R_3SnX in which R is phenyl, has also proinent activity as antifoulants and are used as ship paints and undercoating. Ph_3SnX (X=OH) are used also as agricultural fungicides for potatoes, beans, and beats. On the other hand R_4SnX have only few applications like transformer oil stabilizers.

Toxicity—Acute toxicities of organotin compounds in oral administration almost parallel with biocidal activity, and decrease in the order of $R_3Snx > R_4Sn > R_2SnX_2 > R_2SnX_3$, but in the order of $R_2SnX_2 > R_3SnX$ in intraperitoneal application. This may be related to their metabolism or absorption. R_2SnX_2 type compounds, in which R is alkyl, cause severe irritation to skin, eyes, and mucosa, and local damage and therefore they cause vomitation, diarrhoea and gastrointestinal hemor-

rhage when they are administered orally. Generally, toxicity of alkyl compounds are stronger than those of aryl compounds. Feeding rats with dibutyltin dichloride resulted in injuring of bile duct and a dose-dependent reduction in the weight of thymus which may cause immunological effect. In subacute toxicity test of Bu_3SnCl, which is one of R_3SnX compound, diarrhoea, live necrosis, decrease in feed intake, and haemorrhages were observed. Remakable changes were not observed in rats, which were given feed containing in 50 ppm level of Ph_3SnCl for life, but diarrhoea and decrease in weight were observed. In addition to these, decrease in the number of leukocytes and lymphocytes were observed to some extent. Lethal doses (LD_{50}, oral, mg/kg)[5]: Bu_2SnCl; 100-182, Bu_3SnCl; 129, TBTO; 112-234, triphenyltin acetate (Ph_3SnOAc); 136-491. No-observed-effect level (NOEL) of Bu_2SnCl_2 to rats, by environmental Health Criteria (EHC, WHO) were 1 mg/kg body weight/day, Ph_3Sn^+ (Ph_3SnOH) to guinea pigs were 0.25 mg/kg body weight/day and Ph_3Sn^+ (Ph_3SnOAc) to guinea pigs were 0.1 mg/kg body weight/day. If the safety factor for man is assumed to be 100, acceptable daily intake (ADI) for man of 50 kg body weight is calculated to be 50-500 μg/man (50 kg)/day. Formal ADI published by FAO/WHO is 0.5 μg/kg body weight, i.e. 25 μg/man (50 kg)/day. No formal ADI by FAO/WHO is not reported on TBTO, but 1.6 μg/kg body weight, i.e. 80 μg/man (50 kg)/day have been adopted as a interim ADI in Japan.

THE OBJECT OF THE STUDY

We tried to establish an analytical method for organotin compounds in order to investigate the food contamination by these compounds.

EXPERIMENT

Analysis—Method I (Gel permeation chromatography (GPC) method[2]: Fish sample was homogenized with equal amount (w/v) of 0.5 N HCl-methanol (MeOH), and the homogenate was extracted with hexane (Hex), and the extract was loaded onto the GPC column after concentration. The eluate (methylene chloride/cyclohexane, 1:1) (110-175 m*l*) was alkylated by MeMgBr or Pentyl MgBr after concentration, and the reaction mixture was extracted with Hex. The extract was concentrated to 2 m*l* and injected onto FPD/GC. Method II (Silica gel dry column method)[3]: One weight of the fish samples was homogenized with two weights of MeOH with a Biotron. A 10g portion of NaCl and 50 m*l* of 3 N HCl were added to the homogenate (15g, equivalent to 5g of fish flesh), which was then extracted with two portions of 100 m*l* of ether (Et_2O)-Hex (60:40, v/v). The organic layer was dried on anhydrous (anhyd.) Na_2SO_4 after addition of $NaHCO_3$ (0.1g), and H_2O (0.5 m*l*). After concentration, EtMgBr (3 M, Et_2O soln, 2 m*l*) was added to the residue, and the mixture was allowed to stand for 30 min. The residue was transffered to a 25 m*l* glass tube (2.2 cm i.d.) with a ground-glass stopper with Et_2O (2 m*l*). EtMgBr (3M, Et_2O Soln, 2 m*l*) was carefully added and the solution mixed thoroughly and allowed to stand for 30 min. A portion of Et_2O (2 m*l*) and H_2O (10 m*l*) were then added drop by drop to the solution in an ice bath until violent bubbling ceased after addition of 1-2 m*l* of H_2O. After gentle mixing, solid anhyd. Na_2SO_3 (0.2g) and concentrated (conc.) HCl (2 m*l*) HCl (2 m*l*) were added and the mixture was vigorously shaken. This reaction mixture was extracted with Hex (5 m*l*×2), and the Hex solution was dried over anhyd. Na_2SO_4 and then concentrated to near dryness under reduced pressure. The residue was cleaned up by silica gel dry column method. Method III (Florisil, Sep-Pak Florisil method)[4]: Extraction procedure is the same as described in Method II. The organic layer was dried over anhyd. Na_2SO_4 and concentrated to dryness under reduced pressure below 35°C. The residue was dissolved in a small volume of Et_2O and applied to a Florisil Et_2O (40 m*l*) and then eluted with acetic acid (AcOH)-Et_2O (1:99, v/v, 40 m*l*). Collected eluate was evaporated under reduced pressure at 35°C. Complete removal of the final volume of

AcOH remained in the residue was facilitated by addition of a small amount of Hex (4-5 m*l*). The residue was transferred to a 25 m*l* glass tube (2.2 cm, i.d.) sealed with a ground-glass stopper with Et_2O (2 m*l*), and then ethylated in the same way in Method II. Hex extract was dried over anhyd. Na_2SO_4 and then concentrated under reduced pressure to near dryness. The concentrate was applied to a Sep-Pak cartridge, prewashed with a mixture of Et_2O-Hex (1:99, v/v) and eluted with the same solvent mixture. The first 8 m*l* of eluate was was collected and concentrated to 2 m*l* for FPD/GC. A gas chromatograph, equipped with a flame photometric detector (FPD), was operated in the tin mode with a fused silica capillary column CBP 10 (equivalent to OV-1701, 0.53 mm (i.d.)×12 m). Operating temperature: column oven, programmed from 130°C (hold 4 min) at the rate of 20°C/min to 240°C (hold 5 min); injection port, 240°C; detector, 300°C. Gas flow rates: He carrier gas, 20 m*l*/min; H_2, 150 m*l*; air, 100 m*l*/min. The concentration of tetrasubstituted tin was determined by peak height. The standard solutions for FPD/GC were prepared as described above except that the Sep-Pak Florisil procedure was omitted. Standard solutions were stored in a refrigerator in galss bottles containing a small amount of crystalline Na_2SO_3. Method IV (Morin-postcolumn method)[7]: Fish sample was homogenized with 0.9% NaCl soln. (10 m*l*) and then conc. HCl and NaCl (2g) was added to the homogenate. The mixture was extracted with Et_2O (20 m*l*×2). The extract was dissolved into Hex and purified on silica gel pretreated with HCl (elution with Hex-EtOAc). After evaporation of the solvent the residue was dissolved into Hex, and then subjected to Morin-postcolumn detection method (Unisil CN, 4.6 mm×25 cm, Hex-EtOAc-AcOH, 80:20:5, 0.005% Morin reagent in EtOH).

RESULTS AND DISCUSSION

Analytica method—Method I: The sensitivity and shape of peaks of methylated products of Bu_3Sn^+ and Bu_3Sn^{2+} were compared by using S (394 nm) and Sn filter (610 nm) on the chromatograms of FPD/GC. The use of Sn filter did not improve the sensitivity but did improve the shape of the tetra-alkyltin peaks. Methylation was superior to pentylation in sensitivity and shape of peaks. It was found that the loss of Bu_3SnCl and Bu_3SnMe greatly increases to complete dryness. Addition of Na_2SO_3 (about 0.2g) or NaOH (about 0.2g) to the standard solution was effective for prevention of the storage loss. Collection of 110 – 175 m*l* eluate in GPC was suitable for purpose of removal of fat. Recovery test from fish gave 80-105% recovery. Analysis of 8 Hamachi samples (Yellowtails are called Wakashi, Inada, Hamachi, and Buri in Japan in order of the growth stage), which was raised and collected in 1986 (Nov.), showed that Bu_2Sn^{2+} (as Bu_2SnCl_2 and so on, 0.02-0.11 ppm, mean 0.06 ppm), and Bu_3Sn^+ (as Bu_3SnCl and so on, 0.02-2.04 ppm, mean 0.61 ppm) was found in every sample. Method II: Addition of MeOH improved the recovery of Bu_3Sn^+ from fish sample when the recovery test was compared among the samples homogenized with H_2O, 0.5 N HCl and MeOH. Analyses of Wakashi, Inada, Hamachi, and Buri showed that the residue levels in red muscle are higher than those in white muscle and the concentration ratio of Bu_2Sn^+ to Bu_3Sn^+ in liver increased with their growth stage. This may come from the changes of drug-metabolizing enzyme paralleled with the growth stage. The correlation coefficients between the concentration of Bu_3SnCl and that of Bu_2SnCl_2 in each tissue ranged from 0.864 to 0.991. Samples of white or red muscle or liver obtained from Wakashi and Inada were not over 0.19 ppm (Bu_2SnCl_2 and Bu_3SnCl), but relatively higher values were obtained in Hamachi, i.e. Bu_2SnCl_2: 0.005-0.053 ppm (mean 0.013 ppm, n=4) in white muscle, 0.024-0.225 ppm (mean 0.08 ppm, n=4) in red muscle, 0.195-3.74 ppm (mean 1.17 ppm, n=4) in liver, and Bu_3SnCl: 0.063-1.57 ppm (mean 0.45 ppm, n=4) in white muscle, 0.107-2.39 ppm (mean 0.699 ppm, n=4) in red muscle, 0.078-0.517 ppm (mean 0.517, n=4) in liver. However, only 0.096-0.56 ppm (mean 0.285 ppm, n=3) level of Bu_3SnCl was obtained in Buri, na-

tural yellowtails. Method III: Although the clean up method in Method II is effective for the analysis of Bu_2Sn^{2+} and Bu_3Sn^+, interfering substances appeared for the analysis of Ph_2Sn^+ and Ph_3Sn^+ in some species, so they were removed by passing through Sep-Pak Florisil cartridge. This method is applicable to Bu_3Sn^+, Bu_2Sn^{2+}, and Ph_3Sn^+ (Recovery; 83.8-107.2%) but could not be applicable to Ph_2Sh^{2+} as the recovery of Ph_2Sn^{2+} varied from one sample to another. Analytical results of natural samples (32 species, 52 samples), which were purchased in 1988 (Jun.-Aug.) in Tokyo metropolitan area were nd (not detected)-0.319 ppm (mean 0.062 ppm) of Bu_3SnCl, nd-0.047 ppm (mean 0.005 ppm) of Bu_2SnCl_2, nd-1.45 ppm (mean 0.271 ppm) of Ph_3SnCl. When they were classified by fish species, 0.25 ppm (mean, n=3) of Bu_3SnCl in jack mackerel, 0.172 ppm (mean, n=3) of Bu_3SnCl in sardine, 0.41 ppm (mean, n=3) of Ph_3SnCl in sardine, 0.205 ppm (mean, n=3) of Bu_3SnCl in mackerel, 0.76 ppm (mean, n=3) of Ph_3SnCl in mackerel. It was also found that Bu_3Sn^+ is metabolized to Bu_2Sn^{2+} and its hydroxylated product at a butyl chain, $Busn^{2+}CH_2CH_2CH$ (OH) CH_3, in fish liver.

CONCLUSION

A method for simultaneous determination of Bu_3Sn^+, Bu_2Sn^{2+} and Ph_3Sn^+ in marine product was established. Detection limit of each tin compound was 0.2 ng. Analytical results of raised yellowtails showed that Bu_3Sn^+ and Bu_3Sn^{2+} in red muscle was higher than those in white muscle at every stage of the growth. The concentration ratio of Bu_2Sn^{2+} to Bu_3Sn^+ in liver increased with their growth stage. This suggests that Bu_2Sn^{2+} was not concentrated through absorption from the sea, but derived from the metabolism of Bu_3Sn^+. Although the number of fish studied was limited, the presence of Bu_3Sn^+, Bu_2Sn^{2+} and Ph_3Sn^+ in almost all samples suggests widespread marine pollution. The especially high levels in the popular fish, like mackerel, sardine and jack mackerel, are of concern since these fish are eatable in Japan. This does not necessarily mean the risk by these compound, but it is recommended that polluted area be specified and the cause of pollution be eliminated. The residue level of yellowtail with Bu_3Sn^+ decreased in 1988 compared with that in 1986, but it was made clear that there exist contamination with Ph_3Sn^+ in 1988. It is probable that the use of Bu_3SnCl or TBTO may be replaced by the use of Ph_3SnX. A new metabolite, $BuSn^{2+}$ CH_2CH_2CH (OH) CH_3, was found in raised yellowtails in addition to Bu_2Sn^{2+}. In 1990 (Jan.), Bu_3SnX and Ph_3SnX were assigned as one of the first class specified chemicals, and one of the second class specified chemicals, respectively, depending on "Law on Inspection and Regulation for Production of Chemicals", and their production, import, and use have been regulated like PCB, DDT or other organochlorin pesticides.

REFERENCES

1. Van der Kerk, G.J.M. "Organotin Compounds" *Annual Report of International Meeting on Tin* (1972).
2. Sasaki, K., Ishizaka, T., Suzuki, T. and Saito, Y. *J. Assoc. Off. Anal. Chem.* **71,** 360-363 (1988).
3. Sasaki, K., Suzuki, T. and Saito, Y. *Bull. Environ. Contam. Toxicol.* **41,** 888-893 (1988).
4. Ishizaka, T., Nemoto, S., Sasaki, K., Suzuki, T. and Saito, Y. *J. Agric. Food Chem.* **37,** 1523-1527 (1989).
5. Van der Kerk, G.J.M. and Luijten, J.G.A. *J. App. Chem.* **4,** 314 (1954).
6. *WHO Environmental Health Criteria* **15,** Tin and Organotin Compounds; World Health Organization; Geneva (1980).
7. Yu, T. and Arakawa, Y. *J. Chromatogr.* **258,** 189-197 (1983).

II. PROBLEMS FROM FOREIGN AGRICULTURAL PRODUCTS

INTRODUCTION

While the growth of economy made us confortable, it has caused various kinds of frictions in and among countries. Increasing import of agricultural products by recent sharp appreciation of the yen have increased the pleasure of the table, but on the other hand, it has caused economic problems like pressing heavily upon the life of Japanese farmers. On the other hand, it had become impossible to discuss the problems of pesticide residue only in Japanese territory. One of these problems is post-harvest pesticides. This means the pesticides which are used after harvest as its word say, but there is no practical use in Japan except methylbromide, which is used as a fumigant for protection of stored cereals from fruits fly. About 30 pesticides, which have tolerance and 13 pesticides, which are exempted from tolerance, have been registered for this purpose in USA. Some of these pesticides have a possibility that they directly come onto our table becausee they are not exposed to the weather. On the other hand the use of these pesticides as post-harvest pesticides is not allowed in Japan. Accordingly high level of residues such as in post-harvest procedure have never seen in Japan from the point of Good Agricultural Practice (GAP). I would like to discuss the safty of pesticide laying stress on these examples.

Chlorpropharm (IPC)—IPC is registered in Japan as a herbicide for fruits, vegetables and potatoes, and its Reservation Limit for Registration (RLR) is 0.05 ppm. On the other hand, it is not only used as a herbicide, but also as a antisprout agent for potatoes in USA or EC countries, and its tolerance in USA is 50 ppm, which is 1000 time of Japan. ADI by FAO/WHO is still not decided. IPC was detected in 3/8 samples of freezed potatoes for fried potatoes, which were imported from USA in 1982, and IPC residues in all three samples detected are over Japanese RLR[1]. IPC was also detected in one (0.39 ppm) in 9 samples of potatoes, which were imported from USA in 1989, and two (1.5 and 1.1 ppm) in 2 samples, which were imported from Belqium[2]. Their concentration is over Japanese RLR, but not over USA tolerance. When these potatoes were cooked in vegetable oil, decrease rate of IPC was 55%[1]. On the other hand, daily intake of IPC from food was 0.347 μg/kg body weight/day, and ranked second next to 2-Ethylhexyl diphenyl phosphate in Total Diet Study of USA/FDA in 1981/1982[3].

Organophosphate—Malathion, MEP (Fenitrothion) and Chlorpyrifosmethyl will be picked up as pesticides, which are often found in cereals imported from foreign countries. In the survey by Nagayama *et al.*[4] in 1984/1988, they detected maily Malathion (Detection: 77%, n=35, 1 ppm<13%) in imported wheat from USA. RLR of Environment Agency in Japan is 0.5 ppm. International maximum residue limit (MRL) is 8 ppm. Chlorpyrifosmethyl was found next to it, but the levels were low. MRL for Chlorpyrifosmethyl by FAO/WHO is 10 ppm. Detection of MEP was at extremely low level. Malathion, Chlorpyriforsmethyl and MEP were found in the wheat imported from Canada (n=12, Detection: 17-25%), but the concentration was below 0.1 ppm. Malathion was not found from the wheat imported from Australia (n=11), but MEP was found in every sample and 82% of them were over 1 ppm. The tolerance for MEP in Cereals in Australia is 10 ppm. Chlorpyrifosmethyl (18%) was also found, but the levels was below 0.1 ppm. Malathion (12%) and Chlorpyrifosmethyl (25%) was found in the wheat imported from China (n=8), but their levels were below 0.01 ppm. Residues over 1 ppm are unusual in GAP by pre-harvest application, so that it seems that the pesticides described above were used as post-harvest pesticides for purpose of storage. Here I would like to discuss how much amount of pesticides decrease before they come onto our table.

Gunthur[5] treated wheat with Malathion and checked the residue levels. They found that Malathion decrease with storage time, and its half life is 5.6 months. They also reported storage tem-

perature and water content of wheat have a great influence on the half life.

Alessandrini[6] found that most of Malathion was present in bran and shorts and only small amount was present in flour (10-15%) in milling. Malathion residue in bread, which was made of flour procedure with Malathion, is only 8-16% of the residue in original flour. On the other hand, bran and shorts, which are used as feeds, contained relatively high level of Malthion after a year.

For the purpose of checking the heat-stability, Kawamura *et al.*[7] investigated the decrease of Malathion in flour when it was heated in a electric furnace. The higher the temperature of the furnace was, the more remaining Malathion decreased. Most of Malathion was decomposed or dispersed by heating at 220°C/15 min, and only 6% was remained in flour. On the other hand, Malathion (42%) was remained in flour by heating at 180°C/15 min, but only trace was remained by 30 min heating. Addition of H_2O in flour disturbed the decomposition of Malathion. Kwamura *et al.*[7] also investigated Malathion residues in commercially available wheat flour and wheat flour products in 1979. The results shows that 0.004-0.158 ppm (mean 0.158 ppm, n=15) was found in wheat flour, but not in bread, macaroni, chuka-men and malt drinks, and 0.012 ppm in germ bread, 0.012-0.028 ppm (n=3) in noodle (about 10% amount of wheat flour) and 0.252 ppm in biscuit. The biscuit was made of whole grain, so it contains relatively high level of pesticide residue.

Organochlorine pesticide—U.S. Department of Agriculture prohibited tentatively the circulation of Australin meat in U.S. in 1987 (8/19) since Heptachlor, Dieldrin, DDT, and Chlorpyrifos over tolerance were found in it. So we started to check the residue of organochlorine pesticides and Cholorpyrifos in Australian meat[8]. Heptachlor, Aldrin and Chlorpyrifos were not found in every sample investigated and pp'-DDT in 40/55 of beef samples were below the detection limit. On the other hand, Dieldrin was found in 11/55 of the samples, but only one sample was over the Japanese interim limit, 0.2 ppm. Maximum 0.37 ppm of DDE (n=28) was found in mutton. Higher detection of pp'-DDT was obtained in pork compared with beef or mutton (>0.01 ppm: 15/18), but Heptachlorepoxide, which was found in beef, mutton and horse flesh, was not detected. Contamination of horse fresh (n=19) with pp'-DDT was higher than other cattle, and maximum 0.32 ppm was found. And pp'-DDE was also found in higher frequency. It seemed that pollution with Dieldrin and Heptachlorepoxide was proceeding, but there was no sample over the interim residue limit.

Total diet study—Next let take a glance at Total Diet Study in US, which is the largest country for Japanese import. FDA have enthusuastically conducted Total Diet Study since the 1960's. The results of investigation of pesticides and industrial chemiclas in 1978/1982[3] were as follows. 2-Ethylhexyl diphenyl phosphate and Tributyl phosphate showed relatively high intake from food, but these are plasticizers, not pesticide. Malathion, IPC and 2-Chlorethyl linolate come from post-harvest application. 2-Chlorethyl linolate comes from Ethylene chlorhydrin, which is formed by application of Ethylene oxide for species. pentachlorphenol comes from cereals and meats, but it seems that the cause is in sterilization or wood preservatives of a cow-house or storehouse. Most part of Endsulfan comes from leaf vegetables and fruits. In addition to these chemicals, OPP, total DDT, Toxaphen, Dieldrin, Carbaryl and total BHC were found. Dieldrin is one of the pesticides which ADI is the closest to daily intake, but there still exists 6-7 times difference between ADI and actual daily intake. In addition to it, the survey in Japanese food also showed the same trend.

CONCLUSION

The survey in USA and Japan shows there is no serious problem on food pollution. It seems that most of the problems ever happened come from the pesticides, whcih appeared in the beginning of the history of pesticide. Among thesee pesticides are DDT, Dieldrin, EDB and Daminozide. As many countries still use these pesticides from the regional

or economical reasons, and several hundreds of pesticides and industrial chemicals need analyzing for human safety, more systematic check system will be required. In addition to it, establishment of international MRLs by FAO/WHO, i.e. establishment of international ADIs, and ratification of them are necessary for good international relationships.

REFERENCES

1. Sekita, H., Sasaki, K., Kawamura, Y., Takeda, M. and Uchiyama, M. *Bull. Natl. Inst. Hyg. Sci.* **103,** 127 (1985).
2. Suzuki *et al,* Unpublished data.
3. Gartrel, M.J. *et al. J. Assoc. Off. Anal. Chem.* **69,** 146 (1986).
4. Nagayama, T., Maki, T., Kan, K., Iida, M., Kawai, Y. and Nishima. T. *J. Food. Hyg. Soc. Jp.* **30,** 438 (1989).
5. Gunther, F.A., Lindgren, D.L. and Blinn, R.C. *J. Econ. Entomol.* **51,** 843 (1958).
6. Alessandrini, M.E. *Final Technical Report, U.S. Sept. Agr. Project* No. E15-AMS-8 (a) (1965).
7. Kawamura, Y., Takeda, M., Uchiyama and K., Ishikawa, H. *J. Food Hyg. Soc. Jp.* **21,** 70-74 (1980).
8. Suzuki, T., Sasaki, K., Saito, S. and Fukuda, Y. *J. Food. Hyg. Soc. Jp.* **30,** 48-53 (1989).