Bull. Environ. Contam. Toxicol. (2000) 65:104-111

DOI: 10.1007/s001280000101

Bulletin of Environmental Contamination and Toxicology

# Acute Toxicity to the Golden Apple Snail and Estimated Bioconcentration Potential of Triphenylphosphine Oxide and Series of Related Compounds

C.-C. Lo, T.-T. Hsieh

Agriculture Chemicals and Toxic Substances Research Institute, 11 Kuang Ming Road, Wufeng, Taichung Hsien, Taiwan, Republic of China

Received: 30 July 1999/Accepted: 11 May 2000

Triphenylphosphine oxide is a major byproduct of the Wittig reaction, which is widely used in the synthesis of insect sex pheromones (Anderson and Henrick 1975; Henrick 1977; Lo et al. 1988), and in the synthesis of olefins (Hauser et al. 1963). The conventional Wittig reaction entails the reaction of a phosphonium ylide with an aldehyde or a ketone. This olefination method has enjoyed widespread prominence and recognition because of its simplicity and convenience (Hauser et al. 1963).

However, the Wittig reaction produces waste due to its low efficiency and the mass of reagents required. For example, the synthesis of the major component of the sex pheromone of *Spodoptera litura* (F.), *(Z, E)*-9,11-tetradecadien-1-y1 acetate, involves the Wittig reaction of (*E*)-2-pentenal and ylide (Lo et al. 1988). The synthetic efficiency is expressed by the atom utilization, the ratio of the molecular weight of product to the total molecular weight of reagents, and the low atom utilization rate of 36.9% indicated that there was 63.1% atom wasted (Figure 1), and the major byproduct was triphenylphosphine oxide ($Ph_3PO$) with 48.8% (278.1/569.5) of the atom utilization ratio.

Triphenyltin acetate (Figure 2) is a popular molluscicide for the controlling of the golden apple snail *(Pomacea canaliculata* Lamarck), a serious pest to rice plants in Japan (Hirai 1988), Philippines (Adalla and Morallo-Rejesus 1989), and Taiwan. However, its high phytotoxicity, and high bioconcentration make its use in paddy rice field a risk to the aquatic environment. Therefore, its use was banned at the end of 1998 in Taiwan. The structure of $Ph_3PO$ is similar to triphenyltin acetate, except the center tin is replaced by phosphorus phosphor (Figure 1). Thus, the purposes of these studies are: (1). investigates the possibility of using $Ph_3PO$ and series of related compounds for the pesticidal efficacy against snail, and (2). estimates their bioaccumulation potential expressed as BCF which is the ratio of the concentration of a chemical in an organism to its concentration in the environment. If a chemical possesses a log $K_{ow}$ higher than 3, it should be considered to be highly lipophilic and have a significant bioaccumulation potential (Esser 1986). If the BCF value of a chemical is greater than 100, the chemical is considered to be a bioaccumulating compound (Lundgren 1992).

*Correspondence to:* C.-C. Lo

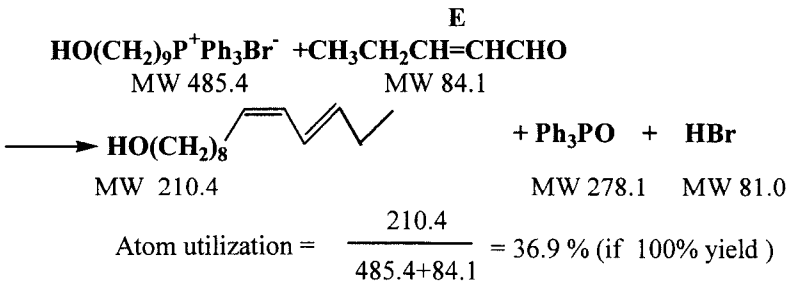


**Figure 1.** The efficiency of atom utilization in the synthesis of (*Z*, *E*)-9,11-tetradecadien-1-ol.

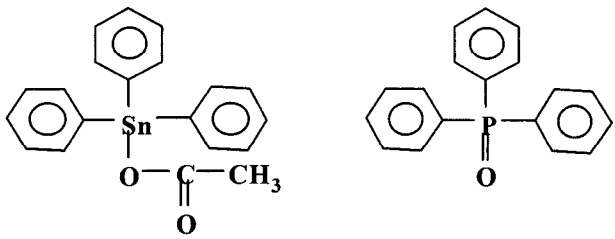


**Figure 2.** Structure of triphenyltin acetate (left) and triphenylphosphine oxide (right).

## MATERIALS AND METHODS

Aquaria with dimensions of 50 cm x 25 cm x 35 cm were used for maintaining and breeding snails in the laboratory. The top of the aquarium was covered with nylon screen. Lamps were positioned 15 cm above the aquaria with a cycle of 16 hrs "on", and 8 hrs "off'. The aquarium floor was covered with a layer of small pebbles, and was filled with water to a depth of 25 cm. Tap water reduced the thickness of the snail shell and the number of eggs, dechlorinated tap water caused the snails to secrete mucus. As a result, fresh aerated well water (pH 7.5, 26 °C) was provided in a continuous flow. Wild adult snails, with a diameter greater than 5 cm, were collected from the field. After copulation, fresh eggs were incubated and the young snails were hatched in 10-17 days. The young snails were fed sweet potato leaves in the aquarium for 65-70 days until maturity. The breeding was repeated and the third generation snails of 35-40 days old were used for toxicity testing.

The acute toxicity of triphenyl compounds against golden apple snails was evaluated by determining the $LC_{50}$ (median lethal concentration) and $LC_{90}$ (90% lethal concentration). The bioassay was conducted in 500 mL glass bottles, each was tilled with thirty snails and 450 mL of water. The concentration of test compounds ranged from 0.5 to 250 $\mu$g/mL (6 different concentrations, three replications) based on their activity, and water without triphenyl compound was used as control. After 72 hrs exposure, the number of dead snails were recorded.

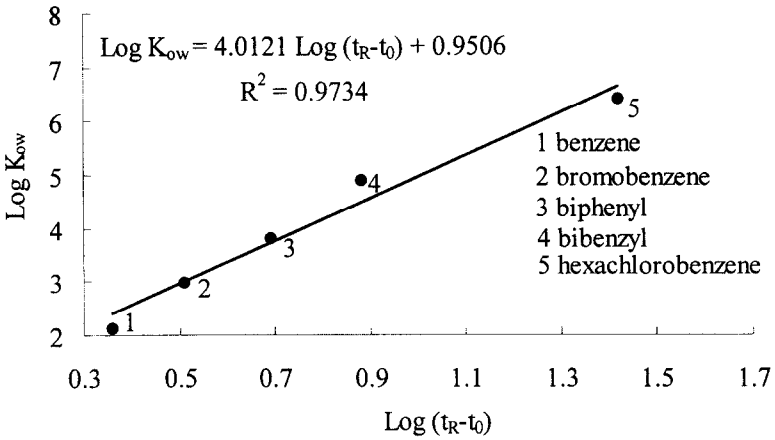


**Figure 3.** Plot of log ($t_R$-$t_0$) versus known log $K_{ow}$ for the reference compounds. Log $K_{ow}$ =a log ($t_R$-$t_0$) + b, where a and b were the slope and intercept, respectively.

The snail was considered to be dead if there was (1) no response to touch, (2) the operculum shut tight, but with no resistance when the operculum was lifted, and (3) the foot was out and swollen. A linear regression was used to calculate the correlation between the concentration (x) and mortality (y).

**Table 1.** Logarithm of the octanol-water partition coefficient and normalized retention time of reference standards.

| Reference standard | Log $K_{ow}$[1] | Log ($t_R$-$t_0$) |
|---|---|---|
| Benzene | 2.13 | 0.36 |
| Bromobenzene | 2.99 | 0.51 |
| Biphenyl | 3.81 | 0.69 |
| Bibenzyl | 4.89 | 0.88 |
| Hexachlorobenzene | 6.42 | 1.42 |

[1]Values of partition coefficient were cited from Eadsforth (1986) calculated from the method developed by Hansch and Leo (1979).

Reference standards of known log $K_{ow}$ values were purchased from Riedel-del Haen (RDH, Wunstorfer, StraBe) (Table 1), and the values of partition coefficient of reference standards were cited from Eadsforth (1986) calculated from the chemical structure using the fragment-addition method of Hansch and Leo (1979). Triphenyl compounds were purchased from Aldrich Chemical Company (St. Louis, MO.) (Table 2). The concentration of triphenyl compounds in *n*-octanol and water was determined by HPLC (Ellgehausen et al. 1981; ASTM 1993). All standards and solvents were reagent grade chemicals. A Shimadzu LC9A equipped with an UV spectrophotometer (SPD-6AV) was used to detect the concentration. A 25 cm x 4.6 mm (I.D.) stainless column packed with Lichrospher 100 RP-18 (5$\mu$m) in LichroCART (Merck) was used.

**Table 2**. Acute toxicity of triphenyl compounds against the golden apple snail, *Pomacea canaliculata* in water

| Compound[1] | Structure (Molecular weight) | $LC_{50}$ μg/mL | $LC_{90}$ μg/mL | Conc. used μg/mL | Linear regression[2] ($r^2$) |
|---|---|---|---|---|---|
| Triphenyltin acetate(97%) | $(C_6H_5)_3SnCO_2CH_3$ (409.1) | 4.8 | 16.1 | 0.5-250.0 | y=3.547x+32.953 (0.887) |
| Triphenyl-phosphine oxide(99%) | $(C_6H_5)_3P(O)$ (278.3) | 12.2 | 29.5 | 4.0-250.0 | y=2.312x+2.897 (0.856) |
| Triphenyl-phosphine sulfide(98%) | $(C_6H_5)_3P(S)$ (294.4) | 16.9 | 33.3 | 5.0-250.0 | y=2.441x+8.742 (0.942) |
| Triphenyl-phosphate(98 %) | $(C_6H_5O)_3P(O)$ (326.3) | 38.2 | 83.5 | 10.0-250.0 | y=0.882x+16.350 (0.834) |
| Triphenyl-amine (98%) | $(C_6H_5)_3N$ (245.3) | 55.0 | 101.3 | 20.0-250.0 | y=0.863x+2.548 (0.960) |
| Triphenyl-phosphine(99 %) | $(C_6H_5)_3P$ (262.3) | 67.4 | 132.2 | 30.0-250.0 | y=0.617x+8.382 (0.932) |
| Triphenyl-methyl mercaptan (98%) | $(C_6H_5)_3CSH$ (276.4) | 87.6 | 150.5 | 40.0-250.0 | y=0.636x+5.732 (0.992) |
| Triphenyl-methane sulfenamide (97%) | $(C_6H_5)_3CSNH_2$ (291.4) | 101.4 | 182.4 | 40.0-250.0 | y=0.493x-0.051 (0.996) |
| Triphenyl-methane (99%) | $(C_6H_5)_3CH$ (244.3) | 95.4 | 174.4 | 40.0-250.0 | y=0.506x+1.702 (0.986) |
| Triphenyl-methanol(97 %) | $(C_6H_5)_3COH$ (260.3) | 140.5 | 235.6 | 40.0-250.0 | y=0.420x-9.063 (0.995) |

[1] Compounds used as received (Aldrich, 97-99%).

[2] x: concentration (μg/mL); y:mortality (%).

The operating conditions were: column temperature, ambient; mobile phase, methanol + water (75:25, by volume); flow rate, 1.0 mL per min.; wavelength, 254 nm. An internal standard solution was prepared by dissolving 2.0 g of acetanilide in 1 L of methanol. The calibration solution was prepared with 200 mg/mL each of 5 reference compounds with the internal standard solution. The internal standard was used to provide a retention time ($t_0$), then the reference standard retention time ($t_R$) could be normalized ($t_R$-$t_0$). A plot of log ($t_R$-$t_0$) versus known log $K_{ow}$ of the reference standards was obtained with the linear regression equation (Figure 3). An estimated log $K_{ow}$ of the triphenyl compounds was calculated from the linear regression. The data of bioaccumulation or bioconcentration factor (BCFs) in snail were calculated from the equation for fish observed by Chiou (1985), and the results were applied for the prediction of

BCF in snail:

Log BCF=0.893 log $K_{ow}$ + 0.607

## RESULTS AND DISCUSSION

Triphenylphosphine oxide was less effective as a molluscicide than triphenyltin acetate (Table 2). The $LC_{50}$ and $LC_{90}$ of triphenylphosphine oxide were 12.2 and 29.5 μg/mL, whereas, the $LC_{50}$ and $LC_{90}$ of triphenyltin acetate were 4.8 and 16.1 μg/mL, respectively. But triphenylphosphine oxide was more toxic than the other triphenyl compounds (Table 2).

The different activity of triphenyl compounds seemed dependent on the nature of center element (X). For example, for triphenyl compound $(C_6H_5)_3XY$ (Y was the secondary functional group attached to center element of X), the toxicity against the snail was mainly influenced by the center element X in the following series: -Sn-($LC_{50}$=4.8 μg/mL, $LC_{90}$=16.1 μg/mL)> –P–($LC_{50}$=12.2-16.9 μg/mL, $LC_{90}$=29.5-33.3 μg/mL)> – C – ($LC_{50}$=87.6-140.5 μg/mL, $LC_{90}$=l50.5-235.6 μg/mL) (Table 2).

By introducing the secondary functional group Y into X, the toxicity is affected slightly. For example, for compound of $(C_6H_5)_3C$ – Y, Y= – SH($LC_{50}$=87.6 μg/mL, $LC_{90}$=150.5 μg/mL)>–$SNH_2$($LC_{50}$=101.4 μg/mL, $LC_{90}$=182.4 μg/mL)> – H($LC_{50}$=95.4 μg/mL, $LC_{90}$=174.4 μg/mL)> – OH($LC_{50}$=140 μg/mL, $LC_{90}$=235.6 μg/mL) (Table 2). For the compounds having no secondary group, the acute toxicity was in the median, the $LC_{50}$ and $LC_{90}$ were 55.0 and 101.3 μg/mL for triphenylamine [$(C_6H_5)_3N$], and 67.4 and 132.2 μg/mL for triphenylphosphine [$(C_6H_5)_3P$], respectively (Table 2).

All compounds showed a good correlation between the concentration (x) and mortality (y) and the coefficient of determination ($r^2$) were in the region of 0.834-0.996 (Table 2). The plot of log ($t_R$-$t_0$) versus known log $K_{ow}$ of the five reference compounds showed a good correlation between the log of normalized retention time (X) and the log of octanol-water partition coefficient (Y). The coefficient of determination ($r^2$) averaged 0.9734 (Figure 3). The log $K_{ow}$'s of the triphenyl compounds were then calculated from the regression analysis, and the results indicated that the log $K_{ow}$ of triphenylphosphine oxide was much lower than the log $K_{ow}$ of triphenyltin acetate by about 2 orders of magnitude (Table 3). Thus, the triphenyltin acetate was considered to be hazardous to the aquatic environment, because its $K_{ow}$ was 25118 (Esser 1986), and BCF was 34372 (Lundgren 1992). The triphenylphosphine oxide was considered to be non-hazardous to the aquatic environment, because its $K_{ow}$ was 20 (Table 3). Other triphenyl compounds were all hazardous compounds because their BCF values were higher than 100 (Table 3).

The mode of action of triphenylphosphine oxide to the golden apple snail is not clear , but the triphenylphosphate was reported as a neurotoxin in animals

**Table 3.** Determination of partition coefficient (log $K_{ow}$) and bioconcentration potential (log BCF, BCF) of triphenyl compounds.

| Compound | $t_R$[1] | $Log(t_R-t_0)$[2] | Log $K_{ow}$[3] | LogBCF[4] | BCF |
|---|---|---|---|---|---|
| Triphenylamine | 25.949 | 1.35 | 6.37 | 6.30 | 1974285 |
| Triphenylmethyl mercaptan | 21.698 | 1.26 | 6.01 | 5.97 | 941738 |
| Triphenylmethane | 21.010 | 1.25 | 5.97 | 5.94 | 867381 |
| Triphenylmethane sulfenamide | 11.524 | 0.91 | 4.60 | 4.71 | 51856 |
| Triphenyltin acetate | 10.615 | 0.86 | 4.40 | 4.54 | 34372 |
| Triphenyl phosphate | 7.415 | 0.61 | 3.40 | 3.64 | 4397 |
| Triphenylmethanol | 7.400 | 0.61 | 3.40 | 3.64 | 4397 |
| Triphenylphosphine sulfide | 7.332 | 0.60 | 3.36 | 3.61 | 4050 |
| Triphenylphosphine | 4.877 | 0.19 | 1.71 | 2.13 | 136 |
| Triphenylphosphine oxide | 4.578 | 0.09 | 1.31 | 1.78 | 59 |

[1]$t_R$: Retention time of triphenyl compound (min.).

[2]$t_0$: Retention time of internal standard, acetanilide ($t_0$=3.343 min.).

[3]Determined by the regression analysis: Log $K_{ow}$=4.0121 log ($t_R$-$t_0$)+0.9506 ($r^2$=0.9734).

[4]Calculated from the equation: Log BCF=0.893 log $K_{ow}$+0.607.

(DHHS, 1981), and trialkyltin compounds were known to cause an edema in the white matter of the brain in mammals (Thayer, 1974). Hansch (1969) pointed out that a variety of neutral drugs acting on the central nervous system have log P value near 2. The partition coefficient (log P or log K,,) of triphenylphosphine oxide was 1.31 and its structure is close to triphenylphosphate. These findings suggested that the possibility of neurotoxicity.

The partition coefficient of triphenylphosphine [$(C_6H_5)_3P$] was 1.71, but its $LC_{50}$ and $LC_{90}$ were 5.5 and 4.5 times less than the toxicity of triphenylphosphine oxide [$(C_6H_5)_3P(O)$], respectively, indicated that an oxidized form was better than a reduced form for higher toxicity (Table 2). The trend of an oxidized form with higher toxicity was also observed in other triphenyl compounds: $(C_6H_5)_3P(O)$, $(C_6H_5)_3S(O)$> $(C_6H_5)_3P$, and $(C_6H_5)_3P(S)$> $(C_6H_5)_3CSH$, $(C_6H_5)_3CSNH_2$ (Table 2). If the phenyl group attached to P was replaced by the phenoxy group, this would reduce the activity.

In conclusion, the different activity of triphenyl compounds seems dependent on the nature of center element (X). By introducing the secondary functional group Y into X, the toxicity is affected slightly. For the compounds having no secondary group, the acute toxicity is in the median. An oxidized form is better than a reduced form for higher toxicity. Triphenylphosphine oxide may be a neurotoxin to the golden apple snail, and triphenylphosphine oxide may be a good alternative to triphenyltin acetate. The acute toxicity of triphenylphosphine oxide ($LC_{50}$=12.2 $\mu$g/mL, $LC_{90}$=29.5 $\mu$g/mL ) is comparable to triphenyltin

acetate ($LC_{50}$=4.8 μg/mL, $LC_{90}$=16.1 μg/mL ). The partition coefficient of triphenylphosphine oxide (log $K_{ow}$=1.31) is lower than the partition coefficient of triphenyltin acetate (log $K_{ow}$=4.4) indicates the risk of bioaccumulation for triphenylphosphine oxide is lower than triphenyltin acetate. The Wittig reaction is very useful in the synthesis for olefins, but the low atom utilization ratio produces a large amount of waste, triphenylphosphine oxide, because this reaction usually starts with a triphenylphosphine and an alkyl halide. The results of these studies may provide an opportunity to integrate waste management, while providing an opportunity to use what is now an unused resource.

## REFERENCES


Adalla CB, Morallo-Rejesus B (1989) The golden apple snail, *Pomacea* sp. a serious pest of lowland rice in the Philippines. British Crop Protect Coun 41: 417-422

Anderson RJ, Henrick CA (1975) Stereochemical control in Wittig olefinsynthesis. Preparation of the pink bollworm sex pheromone mixture, Gossyplure. J American Chem Soc 97: 4327-4334

ASTM (1993) Standard test method for partition coefficient (*n*-octanol/water) estimation by liquid chromatography. Annual Book of ASTM Standards. Philadelphia, USA. Vol. 11.04, E 1147-92, pp. 813-816

Chiou CT (1985) Partition coefficient of organic compounds in lipid-water systems and correlation with fish bioconcentration factors. Environ Sci Technol 19: 57-62

Eadsforth CV (1986) Application of reverse-phase h. p. 1. c. for the determination of partition coefficients. Pestic Sci 17: 311-325

Ellgehausen H, D'Hondt C, Fuerer R (1981) Reversed-phase chromatography as a general method determining octan- l -ol/water partition coefficient. Pestic Sci 12: 219-227

Esser HO (1986) A review of the correlation between physicochemical properties and bioaccumulation. Pestic Sci 17: 265-276

Hansch C (1969) A quantitative approach to biochemical structure-activity relationships. Accounts Chem Res 2: 232-239

Hansch C, Leo A (1979) Substituent constants for correlation analysis in chemistry and biology. John Wiley & Sons, New York.

Hauser CF, Brooks T W, Miles M L, Raymond M F, Butler G B (1963) The Wittig synthesis. I. Use of certain aliphatic aldehydes as intermediates in the synthesis of olefins. J Org Chem 28: 372-379

Henrick CA (1977) The synthesis of insect sex pheromones. Tetrahed Lett 33: 1845-1889

Hirai Y (1988) Apple snail in Japan – The present status and management. JARQ 22: 161-165

Lo CC, Hung MD, Hwang JS, Hung CC (1988) An improved method to synthesize the major component of the sex pheromone of *Spodoptera litura* (F). J Chinese Agric Chem Soc 26: 536-544

Lundgren A (1992) Environmental hazard classification of chemicals. Toxicol Lett 64/65: 535-545

Mackison FW, Stricoff RS, Partridge Jr. LT (1981) Occupational health guidelines for chemical hazards. DHHS (NIOSH) Publication No. 81- 123 U.S. Government Printing Office, Washington, D.C.

Thayer, JS (1974) Organometallic compounds and living organisms. J Organometal Chem 76:265-295