# Photooxidation Mechanism of Fenthion by Singlet Oxygen: Evidence by ESR Analysis with a Selective Spin Trapping Agent

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Our previous study suggested that UVB irradiation of the organophosphorus pesticide, fenthion and its photodegradation product, 3-methyl-4-methylthiophenol (MMTP) yielded fenthion sulfoxide and 3-methyl-4-methylsulfinylphenol (MMSP), and that the formation mechanism was related to generation of singlet oxygen ($^1O_2$). The objective of this study was to elucidate the $^1O_2$-triggered photooxidation mechanism of fenthion in detail. Generation of $^1O_2$ in the photooxidative reaction was directly detected by electron spin resonance (ESR) technique with 2,2,6,6-tetramethyl-4-piperidone (TMPD) as a selective $^1O_2$ spin trapping agent which yields 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TEMPONE). When fenthion and MMTP solutions were irradiated by UVA or UVB light, the TEMPONE signal was observed. However, no signal was detected after exposure of MMSP, dimethyl phosphorothioate or fenthion sulfoxide solutions to the UV light. The production of the signal in fenthion and MMTP solutions was more predominant under UVB irradiation than under UVA irradiation. When the signal was detected in these solutions, fenthion sulfoxide and MMSP were also formed in amounts proportional to the signal intensity. The TEMPONE signal intensity and the formation of these oxidative products were significantly inhibited by the addition of $^1O_2$ scavenger, L-histidine or sodium azide to the reaction medium. The study provided the first direct evidence that $^1O_2$ is generated during photolysis of fenthion and MMTP by UV irradiation. We also proposed its oxidation mechanism of fenthion and MMTP.

**Key words** —— fenthion, 3-methyl-4-methylthiophenol, photooxidation, singlet oxygen, electron spin resonance, 2,2,6,6-tetramethyl-4-piperidone

## INTRODUCTION

The organophosphorus pesticide, fenthion (phosphorothioic acid *O*,*O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl] ester) is widely used as an effective insecticide for the control of fruit flies, leaf-eating larvae, stem borers, bugs and various other insect pests of rice, fruits, vegetables, beets, *etc*.[1–3] The persistence of this compound in the environment is affected by several factors: photolysis,[4,5] biotransformation in plants,[6] metabolism in insect bodies[7] and bacteriological degradation.[8] A previous study showed that aqueous fenthion was easily photodegraded by UV irradiation to form fenthion sulfoxide, 3-methyl-4-methylthiophenol (MMTP), 3-methyl-4-methylsulfinylphenol (MMSP) and dimethyl phosphorothioate. Thus, photolysis may play an important role in the degradation of fenthion in the environment.[9]

Aqueous photolysis mechanisms of fenthion were proposed on the basis of two kinds of reactions, one is pH-dependent photochemical hydrolysis of the phosphorus-*O*-phenyl ester (Fenthion → MMTP + Dimethyl phosphorothioate), and the other is oxidation of sulfide (Fenthion → Fenthion sulfoxide, MMTP → MMSP).[9] This oxidative reaction was indirectly suggested to be triggered by singlet oxygen ($^1O_2$), because the co-addition of $^1O_2$ scavenger, L-histidine or sodium azide resulted in decreased formation of these oxidative products. However, there has been no report that directly demonstrates $^1O_2$ generation in the photolysis of fenthion; thus, such direct detection is essential for understand-

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ing the oxidation mechanism. The electron spin resonance (ESR) technique with 2,2,6,6-tetramethyl-4-piperidone (TMPD) as a selective $^1O_2$ spin trapping agent which yields 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TEMPONE) by $^1O_2$-triggered oxidation is generally used for direct detection of $^1O_2$ generation.[10–14] The objective of this study was to elucidate the photooxidation mechanism of fenthion by $^1O_2$ in detail. We therefore performed ESR analysis using TMPD to detect $^1O_2$ directly which was generated in UV-irradiatied fenthion and its photodegradation products: MMTP, MMSP, dimethyl phosphorothioate and fenthion sulfoxide. We also looked into the relation between generations of $^1O_2$ and formation of the oxidation products.

## MATERIALS AND METHODS

**Chemicals** —— Fenthion (> 98.0% purity), fenthion sulfoxide (> 99.0% purity) and MMTP were purchased from Wako Pure Chemical Industries (Osaka, Japan). TMPD (> 98.0% purity) was from Aldrich (Milwaukee, WI, U.S.A.). Dimethyl phosphorothioate (> 98.0% purity) was the generous gift of Mr. K. Yoshii, the National Institute of Health Sciences, Osaka Branch. MMSP was synthesized using the oxidation reaction of technical-grade MMTP according to the method described by Cabras and Plumitallo[2] with slight modification. Purity of the chemicals was determined by the liquid chromatography (LC)/MS and $^1$H-NMR methods as described previously.[9]

**UVA or UVB Irradiation** —— The reaction mixture (1 ml) containing 50 mM each of fenthion, fenthion sulfoxide, dimethyl phosphorothioate, MMTP or MMSP and 0.1 M potassium buffer (pH 7.4) with or without 0.3 M TMPD as the spin trapping agent was placed in a quartz cell and irradiated with UVA fluorescent light (FL20S-BLB; wavelength from 320 to 400 nm, max at 352 nm) or UVB fluorescent light (FL-20SE; wavelength 280 to 320 nm, max at 315 nm) for 5 min at room temperature. Light intensity on the surface of sample was adjusted to 1.0 mW/cm$^2$ by a UVX digital radiometer and a UVX sensor (UVP, Inc., U.S.A.).

**ESR Analysis** —— Each reaction mixture after UV irradiation was quickly transferred to a flat quartz ESR cuvette (LLC-04B, LABOTEC Co., Ltd., Tokyo, Japan), which was fixed to the cavity (ES-UCX2, JEOL Co., Ltd., Tokyo, Japan) of an ESR spectrometer. ESR spectra of 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TEMPONE) which was yielded from TMPD by $^1O_2$-triggered oxidation were measured with a JEOL JES-TE300-II ESR spectrometer (Tokyo, Japan) 1 min after irradiation of the reaction mixture to correct the reaction effect. The analysis conditions were as follows: modulation frequency, 100 kHz; modulation amplitude, 63 $\mu$T; scanning field, 335.8 ± 5 mT; response time, 0.1 sec; sweep time, 2 min; microwave power, 10.0 mW; microwave frequency, 9.42 GHz; temperature, room temperature.

**Analysis of Fenthion and its Photodegradation Products** —— Irradiated reaction mixture was filled with methanol to a volume of 2 ml. Quantitative analyses of fenthion, fenthion sulfoxide, MMTP and MMSP in the sample solution were performed by high performance liquid chromatography (HPLC) as described previously.[9]

## RESULTS AND DISCUSSION

A previous study showed that formation of fenthion sulfoxide and MMSP as photooxidation products of fenthion and MMTP was strongly inhibited by the addition of $^1O_2$ scavenger, L-histidine or sodium azide.[9] Thus it was indirectly suggested that $^1O_2$ contributes to the photooxidative mechanism of fenthion and MMTP. In this study, $^1O_2$ generation was directly detected using the ESR technique to clarify the photooxidation mechanism of fenthion in detail. TMPD is known to be a selective $^1O_2$ spin trapping agent and yields TEMPONE by $^1O_2$-triggered oxidation. Therefore, the TEMPONE signal generated from fenthion, MMTP, MMSP, dimethyl phosphorothioate and fenthion sulfoxide solution following UVA or UVB irradiation was measured by ESR analysis.

As shown in Fig. 1, the characteristic ESR spectra of three equal-intensity lines of TEMPONE were observed after irradiation of fenthion and MMTP solution by either UVA or UVB light for 5 min. Table 1 shows the signal intensity of TEMPONE generated in the reaction mixture containing fenthion and each of its photodegradation products following this irradiation. Significantly high signal intensity was observed in the solutions of fenthion and MMTP compared with that of the control. However, the intensities in MMSP, dimethyl phosphorothioate and fenthion sulfoxide solution were at the same level as those in the control. These results indicate that $^1O_2$ is generated when fenthion and MMTP so-

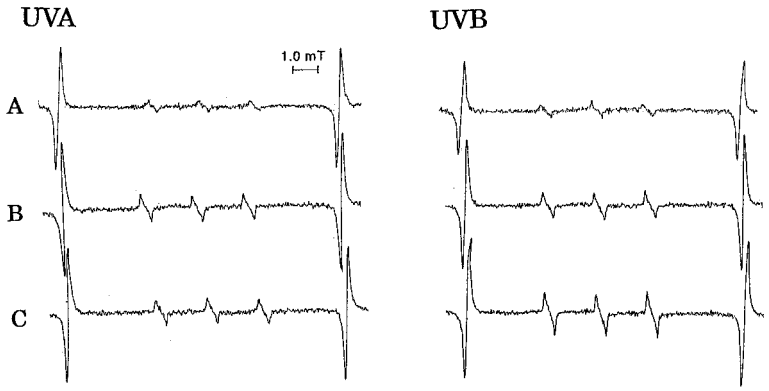


**Fig. 1.** Evidence of $^1O_2$ Formation in UVA or UVB Irradiated Fenthion and MMTP by TEMPONE-ESR Signal

Reaction mixture contained 0.3 M TMPD in 0.1 M potassium buffer (pH 7.4). After the reaction mixture (A), fenthion (B) or MMTP (C) was added and irradiated by UVA or UVB light, and then ESR analysis was performed.

**Table 1.** Formation of $^1O_2$ in the Reaction Mixture Containing Fenthion or its Photodegradation Products Following UVA and UVB Irradiation

| Addition | Relative TEMPONE signal intensity[a)] | |
|---|---|---|
| | UVA | UVB |
| None (control) | 1.0 | 1.0 |
| Fenthion | $2.5 \pm 0.35$* | $2.8 \pm 0.29$* |
| MMTP | $2.2 \pm 0.31$* | $3.8 \pm 0.41$* |
| MMSP | $0.8 \pm 0.12$ | $0.8 \pm 0.16$ |
| Dimethyl phosphorothioate | $0.8 \pm 0.10$ | $1.0 \pm 0.29$ |
| Fenthion sulfoxide | $0.8 \pm 0.17$ | $0.9 \pm 0.15$ |

Reaction mixture containg fenthion, MMTP, MMSP, dimethylphosphorothioate or fenthion sulfoxide was irradiated by UVA or UVB light. *a*) TEMPONE signal intensity was expressed as the ration of signal area of adduct to that of $Mn^+$ at the lowest magnetic field. The relative intensity was calculated by dividing the signal of control value. Data were shown as mean $\pm$ S.D. of three samples with statistically significant differences compared with the control (*$p < 0.05$).

lutions are irradiated by UVA or UVB light, with the $^1O_2$ generation by UVB irradiation being more efficient.

As shown in Fig. 2, the addition of $^1O_2$ scavenger, L-histidine or sodium azide in the reaction medium effectively prevented the signal that was generated by irradiation of both UV lights. These ESR observations provide the definitive evidence that during the photolysis of fenthion by UV, $^1O_2$ is generated from dissolved oxygen in the presence of the parent compound and MMTP as the photooxidative product.

Earlier findings suggested that dissolved oxygen contributed to the generation of $^1O_2$ in the photooxidative reaction of fenthion.[9)] $^1O_2$ is known to be produced when dissolved oxygen is irradiated by short-wave UV light having a wavelength of less than 200 nm; the reaction does not proceed under irradiation of the oxygen by UVA or UVB because dissolved oxygen cannot be excited at a UV wavelength greater than 280 nm.[15)] Moore *et al.*[16)] reported, however, that $^1O_2$ was generated by UVA irradiation of dissolved oxygen in aqueous solution when a photosensitizer such as nalidixic acid or oxolinic acid was added. Therefore, fenthion and MMTP seem to act as photosensitizers in the photooxidative reaction.

The effects of $^1O_2$-scavenger on the formation of fenthion sulfoxide and MMSP in fenthion or MMTP solution by the irradiation were of interest. As shown in Table 2, UVA and UVB irradiation of fenthion and MMTP caused the formation of their oxidative products, in amounts proportional to their TEMPONE signal intensity (Table 1). Furthermore, when $^1O_2$ generation was diminished by the addition of $^1O_2$ scavengers to the reaction mixture (Fig. 2), formation of these oxidative products was significantly inhibited (Table. 2). These results

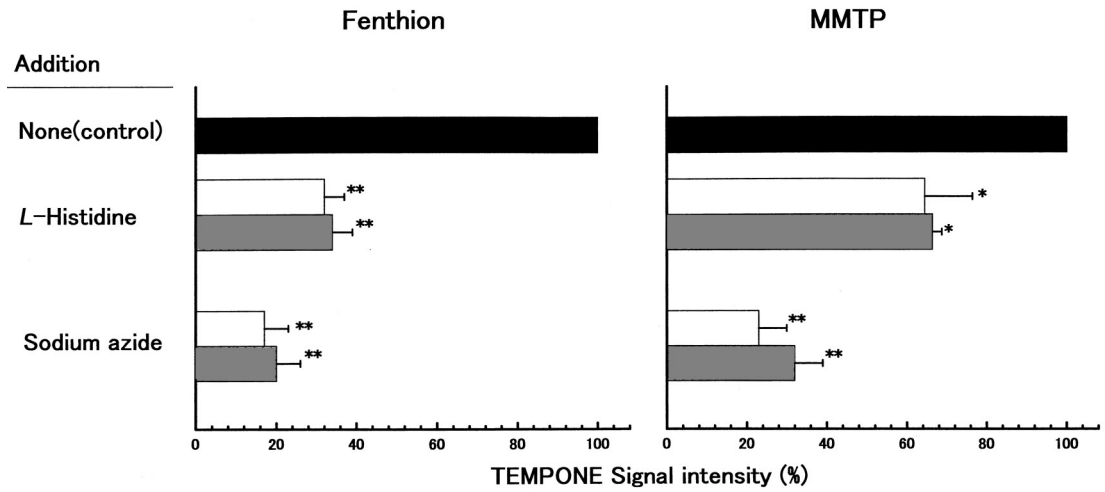


**Fig. 2.** Effect of $^1O_2$-Scavengers on the Formation of $^1O_2$ in Fenthion or MMTP Solution by UVA or UVB Irradiation

Each reaction mixture containing 50 mM fenthion or 50 mM MMTP, 0.3 mM TMPD and 100 mM L-histidine or 100 mM sodium azide was irradiated with UVA (□) or UVB (▨) light for 5 min. TEMPONE signal in each mixture was measured by ESR. The intensities were expressed as mean ± S.D. of three samples with statistically significant differences compared with the control at $p < 0.05$(*) and $p < 0.01$(**).

**Table 2.** Effect of $^1O_2$-Scavenger on the Formation of Fenthion Sulfoxide and MMSP in Fenthion or MMTP Solution by UVA or UVB Irradiation

| | Formation of photooxidation products mM (%) | | | |
|---|---|---|---|---|
| | Fenthion sulfoxide | | MMSP | |
| Addition | UVA | UVB | UVA | UVB |
| None (control) | 3.1 ± 0.9 (100) | 4.3 ± 1.4 (100) | 6.9 ± 1.4 (100) | 8.2 ± 3.1 (100) |
| L-histidine | 0.4 ± 0.1* (13) | 0.8 ± 0.2* (19) | 2.0 ± 0.5* (29) | 2.2 ± 0.6* (27) |
| Sodium azide | 0.1 ± 0.1* (3.2) | 0.2 ± 0.1* (4.6) | 0.5 ± 0.2* (7.2) | 0.5 ± 0.1* (6.1) |

The initial concentration of each fenthion and MMTP was 50 mM. Fenthion sulfoxide and MMSP were formed from fenthion and MMTP, respectively. After fenthion or MMTP solution was irradiated by UVA or UVB light with or without (control) L-histidine or sodium azide for 5 min, concentration of fenthion sulfoxide and MMSP were determined. The figure in parentheses are the percentage of each productive concentration for control. Percentage was calculate on the basis of the average value. Data were shown as the mean ± S.D. of three samples with statistically significant differences compared with the control at $p < 0.01$(*).

clearly indicate that $^1O_2$ contributes to the photooxidation of fenthion to fenthion sulfoxide and of MMTP to MMSP. Notice that since TMPD competes with L-histidine and sodium azide in reaction with $^1O_2$, the inhibition effects of these scavengers estimated by the ESR measurements (Fig. 2) were much lower than those by the distribution analysis of the oxidized products (Table 2).

Both fenthion and MMTP have two-coordinative sulfide in their molecules. Since the sulfur atom in this sulfide has two lone pairs, the sulfide easily undergoes nucleophilic attack by $^1O_2$ as a kind of electrophilic reagent and forms sulfoxide.[17] Actually, Foote and Peter[18] and Jensen and Foote[19] indicate that sulfoxide [$R_2SO$] is formed by the nucleophilic attack of $^1O_2$ to sulfide [$R_2S$] and the reaction proceeds via a persulfoxide [$R_2S^+OO^-$] as the intermediate. Thus we suggest that the oxidation of fenthion and MMTP to form their sulfoxides proceeds by nucleophilic attack of $^1O_2$ to sulfide, and we therefore propose the oxidation mechanism of fenthion and MMTP shown in Fig. 3.

$^1O_2$ is generated from dissolved oxygen in the photochemical reaction of fenthion and MMTP solution under UVA or UVB irradiation, and reacts with the methylthio group in their compounds, yielding fenthion sulfoxide and MMSP through the intermediate persulfoxide.

In a conclusion, we suggest that $^1O_2$ has a major contribution to the photolysis of fenthion in a water environment.

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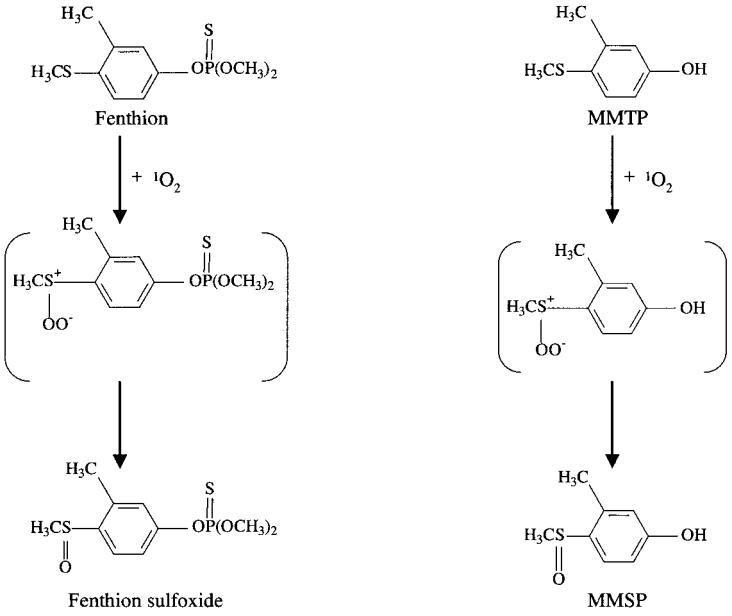


**Fig. 3.** Proposed $^1O_2$-Triggered Oxidation Mechanism of Fenthion to Fenthion Sulfoxide and MMTP to MMSP

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