Thermal Stability of Organophosphorus Pesticide Triazophos and Its Relevance in the Assessment of Risk to the Consumer of Triazophos Residues in Food

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The degradation of triazophos in aqueous solutions was monitored at 205 and 254 nm after separation using high-performance liquid chromatography. An ODS column was used with a mobile phase of 60% acetonitrile and 0.04% phosphoric acid at a flow rate of 1.4 cm^3 min^{-1}. When dissolved in distilled water, ~30% of the original triazophos was detected. The effect of heating time and temperature on a 0.5 mg dm^{-3} standard was investigated. Over a 150 min period at 100 °C the peak area detected for the standard decreased by 58.67 ± 6.19 and 65.03 ± 4.61% when measured at 254 and 205 nm, respectively. The precision of the absorbance detected at 205 and 254 nm was 3.54 ± 2.8 and 3.86 ± 3.9%, respectively. There was a significant difference ($P = 0.10$) between the precision of the results obtained at each wavelength. The t_{calcd} value was −2.236 and the t_{crit} value was 1.94. The most sensitive wavelength was 205 nm. A 54% difference in the gradients of the calibration graphs obtained at each wavelength was observed. The results suggest that ~72% of triazophos is degraded during a 20 min cooking period at 100 °C, due to ambient and elevated temperature hydrolysis. Therefore, the dose to the consumer of triazophos residues in cooked food is likely to be ~72% lower than in the raw food, with a concomitant reduction in toxicological risk.

Keywords: *Triazophos; cooking; stability; high-performance liquid chromatography; ultraviolet detection; organophosphorus pesticides; risk to the consumer; pesticide residues in food*

INTRODUCTION

There is growing concern about residues of xenobiotics in food. Pesticides are of particular concern because they generally have high toxicity profiles. Despite this, the risk to the consumer from residues in food in countries where good pesticide control legislation is in operation is very low. However, from time to time unacceptably high residues of a particular pesticide occur in food, and questions are asked about the potential impact of such an event on consumers [e.g., triazophos in apples in the United Kingdom (MAFF, 1998)]. Generally, risk assessments are based on residue levels in uncooked food, even though a large proportion of food consumed is cooked or processed in some other way (e.g., cured) before being eaten. To properly assess the risks of pesticide residues to the consumer, it is necessary to consider the effects of cooking upon the residues. In the present study, we report the effects of heating aqueous solutions of the organophosphorus pesticide (OP) triazophos as a means of mimicking the cooking process and so provide data to assist in more accurately assessing the risk of pesticide residues to the consumer.

Triazophos (CAS Registry No. 24017-47-8) is the common name for *O*,*O*-diethyl *O*-1-phenyl-1*H*-1,2,4-triazol-3-yl phosphorothioate (Figure 1). It was introduced into agriculture by Hoechst AG in the late 1970s.

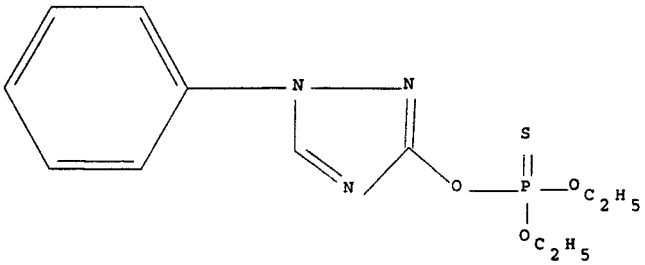

Figure 1. Structure of triazophos.

It is a broad spectrum nonsystemic contact insecticide approved in the United Kingdom for treatment of a broad array of crops including apples, cereals, sweet corn, beans, carrots, and parsnips (Whitehead, 1995). Its maximum residue levels (MRL) range from 1 mg kg^{-1} in carrots and parsnips to 0.1 mg kg^{-1} in Brussels sprouts and cabbage to 0.05 mg kg^{-1} in onions and potatoes (Whitehead, 1995).

Triazophos has been found as residues in a wide variety of foods as part of European and U.S. food monitoring schemes [e.g., in the United Kingdom (MAFF, 1999)]. It has attained worrying residue levels in carrots (MAFF, 1998) and is of concern in apples because they are commonly fed to infants and children [for whom there is a low acceptable daily intake (ADI) for triazophos], either following home preparation or as a component of convenience baby and infant foods (e.g., apple-based bottled infant foods). The effects of cooking on residues of triazophos are therefore of significant importance in determining the risk to consumers of cooked triazophos residue-containing fruits and vegetables.

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Triazophos has vapor pressures of 1.33, 13.3, and 133 mPa at 38, 55, and 74 °C, respectively, its melting point is between 2 and 5 °C, and it has an indeterminable boiling point because it decomposes at 140 °C. It is sparingly soluble in water (30−40 mg L^{-1} at 20 °C) and very soluble in organic solvents (acetone, 1 kg L^{-1}; hexane, 9 kg L^{-1}). Its octanol/water partition coefficient (log P_{ow}) is reported to be found between 3.34 (Roberts and Huston, 1999) and 3.55 (MAFF, 1993).

The effects of cooking on OPs have been reported previously (Nagayama, 1996; Hassan et al., 1993; Coulibaly and Smith, 1993, 1994). Coulibaly and Smith (1993) studied famphur, fenthion, parathion, stirofos, chlorpyrifos, and ronnel in aqueous solutions that were unheated, heated to 70 °C for 1 or 2 h, or heated to 80 °C for 1 h. Stirofos and famphur were largely unaffected by heating. However, fenthion and parathion were hydrolyzed by 55.4 and 53.2%, respectively, in unheated water. Interestingly, heating did not result in further degradation. Chlorpyrifos and ronnel were degraded by 80 and 75%, respectively, in unheated water. On heating, chlorpyrifos degraded by ∼89% when heated for 1 h at 70 °C and by ∼87% when heated to 80 °C for 1 h. It is therefore clear that selected OPs are unstable to heating in aqueous solution, and therefore such OP residues in food would also be expected to degrade on cooking.

The OP triazophos was selected for our studies because it has recently (MAFF, 1998) been found at high levels in fruit and vegetables sampled in the United Kingdom as part of the food surveillance programs.

The methods used for the analysis of triazophos include high-performance liquid chromatography with diode array detection (HPLC-DAD) (Martinez Vidal et al., 1996; Parrilla et al., 1993, 1997; Martinez Galera et al., 1997; Gil Garcia et al., 1997; Garrido Frenich et al., 1997) and gas chromatography with either flame photometric detection (Simplico et al., 1999) or a nitrogen−phosphorus detector (GC-FPD or GC-NPD, respectively) (Valor et al., 1997). The HPLC-DAD methods have used detection wavelengths of 200 (Martinez Vidal et al., 1996; Martinez Galera et al., 1997), 210 (Parrilla et al., 1997; Garrido Frenich et al., 1997), 212 (Parrilla et al., 1993), 220 (Gil Garcia et al., 1997), 245 (Martinez Vidal et al., 1996), and 350 nm (Parrilla et al., 1993). Usually a C18 column has been used, producing recoveries of between 83 and 112.8%. The GC-FPD and GC-NPD methods have used solid phase membrane extraction with fibers coated with either poly(dimethylsiloxane) or polyacrylate. Detection limits ranged from 0.003 to 0.014 μg dm^{-3} for SPME-GC-FPD (Simplico et al., 1999) to 0.43 μg dm^{-3} with HPLC-DAD (Martinez Vidal et al., 1996).

The work reported in this paper investigated the influence of temperature on the degradation kinetics of triazophos in aqueous solution and considers the influence that degradation might have on risk assessment to the consumer. An HPLC method with UV detection was used.

EXPERIMENTAL PROCEDURES

An HPLC pump (Speck Analytical, SA102) was used with a 5 μm, 250 × 4.6 mm, ODS column (Hypersil). A mobile phase of acetonitrile, water, and phosphoric acid was used in the ratio 60:40:0.04, respectively, at a flow rate of 1.4 cm^3 min^{-1}. The injection volume was 25 μL. A Waters 486 tuneable absorbance detector (Millipore) was set at 205 and 254 nm. The wavelengths were monitored in sequential mode.

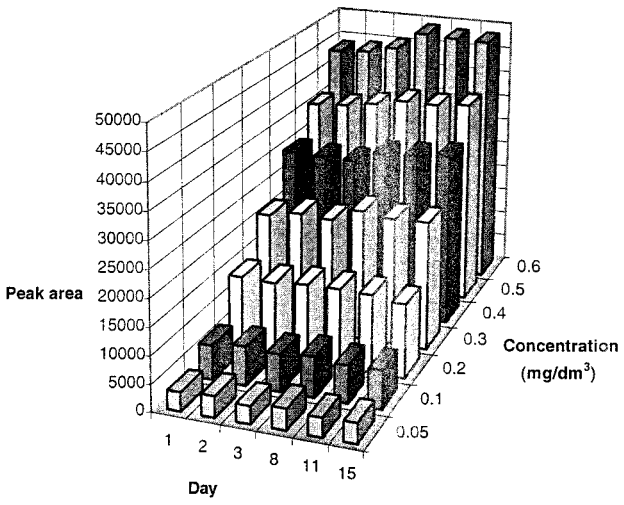

Figure 2. Comparison of the analytical precision achieved at 254 nm ($n = 4$).

A triazophos standard (75% pure, QMx Laboratories, Saffron Walden, Essex, U.K.) was diluted with acetonitrile to prepare working standards in the concentration range of 0.05−0.6 mg dm^{-3}. Standards were analyzed in quadruplicate (Figure 2). Suspected outliers were tested using Dixon's Q-test (Miller and Miller, 1988) at a 95% confidence level.

Extraction Procedure. Sodium chloride (10 g) was added to a 100 cm^3 triazophos standard prepared in acetonitrile. Approximately 13 cm^3 of the acetonitrile layer was transferred to a 15 cm^3 graduated centrifuge tube, 3 g of sodium sulfate was added, the tube was capped, and the contents were shaken well and then centrifuged for 5 min. An aliquot (10 cm^3) was transferred to a 15 cm^3 tube and evaporated to 0.5 cm^3 under clean nitrogen on a water bath at 35 °C. The resulting concentrate was transferred to a conditioned ENVI-CARB SPE tube (Supelco) and eluted with 20 cm^3 of an acetonitrile/toluene (3:1) solution. The eluate was concentrated to ∼2 cm^3 using a rotary evaporator. The concentrate was then solvent exchanged using 2 × 10 cm^3 acetonitrile, evaporating the sample to 2 cm^3 after each addition of acetonitrile. The resulting sample was transferred quantitatively to a 10 cm^3 volumetric flask and bought to volume with acetonitrile.

Stability Studies. A 0.5 mg dm^{-3} triazophos standard solution was prepared and divided equally among six sample bottles. The bottles were then placed in separate water baths at 30, 40, 60, 80, and 100 °C. Watch glasses were placed on the top of the beakers to provide a lid for the beaker. Aliquots of the samples at each temperature were removed at 30, 60, 90, 120, and 150 min. The samples were cooled to room temperature prior to analysis by HPLC-UV with detection at 254 nm. The results at 100 °C were measured at 254 and 205 nm.

RESULTS

Calibration Graph. The long-term stability of triazophos standards was measured by preparing standards (0.05−0.6 mg dm^{-3}) in acetonitrile and analyzing them over a 15 day period. The results are shown in Figure 2 and Table 1. Identical standards were measured at 205 and 254 nm. Linear graphs were obtained at both wavelengths for the concentration range of 0.05−0.6 mg dm^{-3}. At 205 nm the calibration line was $y = 0.1092x + 0.4917$ ($r^2 = 0.9679$), and at 254 nm a less sensitive calibration line was recorded, $y = 0.1209x + 0.4728$ ($r^2 = 0.9593$). The precision at each wavelength is compared in Table 2. The detection limit was 0.035 mg dm^{-3} ($n = 10$) at 254 nm and 0.013 mg dm^{-3} ($n = 4$) at 205 nm.

Stability Studies. When 0.5 mg dm^{-3} triazophos was dissolved in distilled water, the concentration measured

Table 1. Variation in Analytical Precision (Percent) of Standards Analyzed over 15 Days (n = 4)

concn (mg dm^{-3})	day (n = 4) 1	2	3	8	11	15	overall σ
0.05	8.61	6.50	11.44	9.40	7.25	12.39	23.27
0.1	9.37	9.74	1.37	8.20	7.12	2.93	17.64
0.2	3.97	6.71	4.68	6.22	4.38	3.96	12.50
0.3	2.23	2.40	2.38	9.23	2.29	0.80	10.37
0.4	0.69	3.06	1.67	3.39	0.82	1.07	5.09
0.5	1.17	3.44	1.10	2.83	1.17	1.46	5.09
0.6	1.03	2.39	2.17	1.61	2.02	2.96	5.19

Table 2. Comparison of the Analytical Precision (Percent) Achieved at 205 and 254 nm (n = 4)

wavelength (nm)	triazophos concn (mg dm^{-3}) 0.05	0.1	0.2	0.3	0.4	0.5	0.6	overall σ
205	9.48	3.44	0.56	3.80	2.28	3.11	2.11	11.65[a]
254	11.17	7.01	3.38	2.94	1.29	0.62	0.66	14.01[a]

[a] The overall precision is reduced to 6.77 and 8.47% for 205 and 254 nm, respectively, when the 0.05 mg dm^{-3} results are omitted.

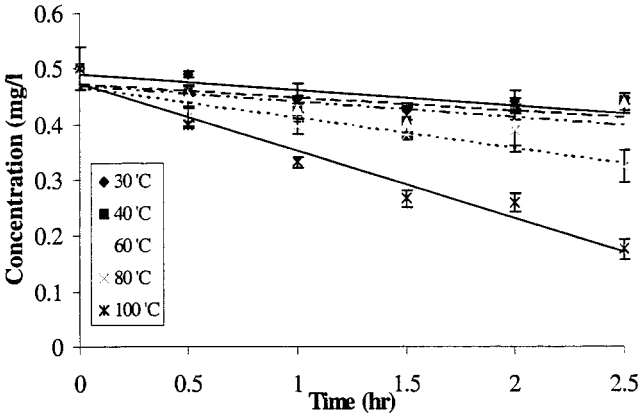

Figure 3. Effect of temperature on stability of 0.5 mg dm^{-3} triazophos measured at 254 nm.

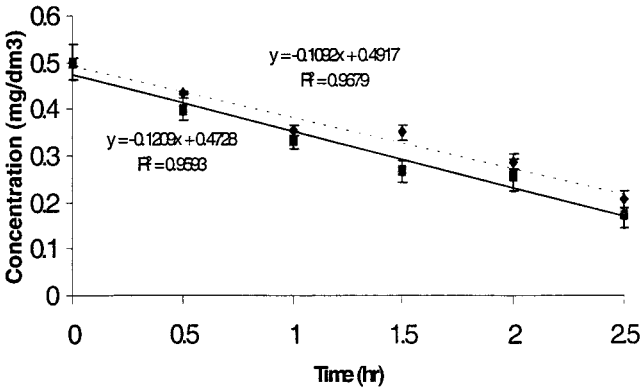

Figure 4. Rate of degradation of 0.5 mg dm^{-3} triazophos at 100 °C as measured at 205 nm (diamonds) and 254 nm (squares).

was 0.135 mg dm^{-3}, which represents a 73% loss due to hydrolysis; at 20 mg dm^{-3} triazophos, the loss due to hydrolysis was 73%.

The effect of temperature and length of exposure on the concentration (0.5 mg dm^{-3}) of triazophos detected is shown in Figures 3 and 4. The 100% value is for an aqueous solution of triazophos at time = 0 min and room temperature. As suspected, there was an increase in the rate of degradation of triazophos with an increase in temperature (Figure 3). The difference in the peak area from time = 0 min to time = 150 min was 14, 12, 13, 36, and 66% for 30, 40, 60, 80, and 100 °C, as detected at 254 nm.

The values obtained for triazophos boiled at 30 °C for up to 2.5 h are relatively constant, decreasing by 2% in the first 30 min and then only a further 9% over the remaining 2 h. The results obtained at 40 and 60 °C were similar, although at 60 °C triazophos reduced by 10% in the first 30 min. The degradation at 80 °C was more pronounced, with 18% of triazophos lost within the first 30 min and then a further 18% degraded in the remaining 2 h. At 100 °C, the peak area for triazophos diminished by 20% within the first 30 min, and this was followed by a steady degradation until only 35% remained after 2.5 h of cooking. From these results, an aqueous sample of triazophos at room temperature would be hydrolyzed by 73%; and on heating at 80 °C for 20 min there would be a further 4% degradation.

The results for 100 °C were also analyzed at 205 nm. The peak area at time = 150 min was 41 ± 7% of the peak area at time = 0 min when measured at 205 nm, whereas the peak area at time = 150 min was 35 ± 5% of the peak area at time = 0 min when measured at 254 nm. The results detected at 254 and 205 nm are compared in Figure 4.

DISCUSSION

Initial method development studies using HPLC-UV investigated the difference between absorbance detection at 254 and 205 nm. The detection limits (3σ) differed between the two wavelengths, with 205 nm proving to be the more sensitive wavelength, although 254 nm gave more precise results.

Triazophos is susceptible to hydrolysis. Degradation in excess of 70% occurred when triazophos was added to water. Continued degradation of the remaining triazophos in aqueous solution resulted in a further 13% hydrolysis following heating at 30 °C for 2.5 h to 65% after 2.5 h at 100 °C (Figure 3). This resulted in total degradations of 90% at 100 °C and 74% at 30 °C. These results are in agreement with Coulibaly and Smith (1993), who showed that selected OPs (not including triazophos) were readily hydrolyzed by 75−87% in unheated water and that heating resulted in only a small increase in hydrolysis.

Most fruits and vegetables are cooked for relatively short times (∼20 min) by boiling in water. For this reason, to assess the potential effect of cooking on the stability of triazophos residues in fruits and vegetables, the degradation values following 20 min of heating at 100 °C have been used. The total triazophos degradation (i.e., ambient hydrolysis plus elevated temperature hydrolysis) after 20 min at 100 °C in water is ∼72%. This figure is a combination of a loss of an initial 70% degradation through ambient hydrolysis and then a further 8% degradation of the remaining 30% (of triazophos), which was subjected to subsequent elevated temperature hydrolysis (20 min at 100 °C); this is equivalent to a further 2.4% loss.

These results suggest that there would be a significant decrease in triazophos residues consumed when compared with residues measured in fresh fruits and vegetables. However, assessing the reduced risk is difficult because it is not possible to determine whether the residue measured in food samples represented the initial total residue level or the residue concentration following spontaneous “cold” hydrolysis. This controversy might explain the recently demonstrated vari-

ability in pesticide residues found in fruits and vegetables extracted by different methods [i.e., the chemistry of the different extraction methods might influence the pesticides' hydrolysis rates (Hamey, 1999)].

In terms of risk to the consumer from residues of triazophos in fruits and vegetables, at best the residues consumed after cooking would be ~72% lower than the measured residue. For example, for apples the highest residue level determined in a study reported by Hamey et al. (1999) was 0.81 mg kg^{-1} [MRL = 0.2 mg kg^{-1}, ADI = 0.001 mg (kg of bw^{-1})]. This means that for a 60 kg human, 0.06 mg of triazophos per day can be consumed safely (i.e., = ADI). This equates to 74 g of apple. An apple weighs ~100 g, and therefore at this residue level it would not be "safe" to consume the entire apple. However, if the residue cooking degradation factor for triazophos is applied, it would be "safe" to consume ~264 g of cooked apple (i.e., approximately three apples). Therefore, even at this very high residue level there would be a very low risk to the consumer when cooking degradation is taken into account.

From these studies it is clear that it is important to consider the effect of pesticide residue degradation on cooking when the risk to the consumer of pesticides in fruits and vegetables that are normally eaten cooked is assessed.

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LITERATURE CITED

(1) Coulibaly, K.; Smith, J. S. Thermostability of organophosphate pesticides and some of their major metabolites in water and beef muscle. *J. Agric. Food Chem*. **1993**, *41*, 1719−1723.
(2) Coulibaly, K.; Smith, J. S. Effect of pH and cooking temperature on the stability of organophosphate pesticides in beef muscle. *J. Agric. Food Chem*. **1994**, *42*, 2035−2039.
(3) Garrido Frenich, A.; Martinez Vidal, J. L.; Parrilla, P.; Martinez Galera, M. Resolution of folpet, procymidone and triazophos in high performance liquid chromatography−diode array detection by using partial least squares calibration to cross-sections of spectrochromatograms. *J. Chromatogr. A* **1997**, *778*, 183−192.
(4) Gil Garcia, M. D.; Garrido Frenich, A.; Martinez Vidal, J. L.; Martinex Galera, M.; Munozdela Pena, A.; Salinas, F. Resolution of overlapping peaks in HPLC with diode-array detection by application of partial least-squares calibration to cross-sections of spectrochromatograms. *Anal. Chim. Acta* **1997**, *348*, 1−3, 177−185.
(5) Hamey, P. V.; Harris, C. The variation of pesticide residues in foods and vegetables, and the associated assessment of risk. *Regul. Toxicol. Pharmacol.* **1999**, *30*, S34−S41.
(6) Hassan, I. M.; Abdallah, M. A.; Naguib, M. M.; Abou Donia, M. A. Toxicity, distribution, accumulation and cooking loss of malathion in tissues of tilapia and common carp fishes. *Grasas Aceites* **1993**, *44*, 339−344.
(7) MAFF. Food and Environment Protection Act 1985, Part III, Control of Pesticide Regulations 1986, Evaluation Triazophos, Nov 1993.
(8) MAFF. Annual Report of the Working Party on Pesticide Residues−1997. MAFF Publications: London, U.K., 1998.
(9) MAFF. Annual Report of the Working Party on Pesticide Residues−1998. MAFF Publications: London, U.K., 1999.
(10) Martinez Galera, M.; Martinez Vidal, J. L.; Garrido Frenich, A.; Gil Garcia, M. D. Evaluation of multiwavelength chromatograms for the quantification of mixtures of pesticides by high-performance liquid chromatography-diode array detection with multivariate calibration. *J. Chromatogr. A* **1997**, *778*, 139−149.
(11) Martinez Vidal, J. L.; Parrilla, V. P.; Galera, M.; Garrido Frenich, A. Cross-sections of spectrochromatograms for the resolution of folpet, procymidone and triazophos pesticides in high-performance liquid chromatography with diode-array detection. *Analyst* **1996**, *121*, 1367−1372.
(12) Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry*, 2nd ed.; Ellis Horwood: Chichester, U.K., 1988; p 62.
(13) Nagayama, T. Behaviour of residual organophosphorus pesticides in foodstuffs during leaching or cooking. *J. Agric. Food Chem*. **1996**, *44*, 2388−2393.
(14) Parrilla, P.; Martinez Vidal, J. L.; Fernandez Alba, A. R. Optimization of the separation, isolation and recovery of selected pesticides in water samples by solid-phase extraction and HPLC photodiode array detection. *J. Liq. Chromatogr*. **1993**, *16*, 4019−4029.
(15) Parrilla, P.; Martinez Galera, M.; Garrido Frenich, A.; Martinez Vidal, J. L. Determination of folpet, procymidone, and triazophos in groundwater by HPLC using partial least squares and principal component regression. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20*, 425−442.
(16) Roberts, T., Huston, D., Eds. *Metabolic Pathways of Agrochemicals. Part Two: Insecticides and Fungicides*; The Royal Society of Chemistry: Cambridge, U.K., 1999; p 508.
(17) Simplico, A. L.; Boas, L. V. Validation of a solid-phase microextraction method for the determination of organophosphorus pesticides in fruits and fruit juice. *J. Chromatogr. A* **1999**, *883*, 35−42.
(18) Valor, I.; Molto, J. C.; Apraiz, D.; Font, G. Matrix effects on solid-phase microextraction of organophosphorus pesticides from water. *J. Chromatogr. A* **1997**, *767*, 195−203.

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